THE UNIVERSITY OF ALBERTA

"THE PENETRATION AND MIGRATION OF SERRATIA MARCESCENS IN PETROLIFEROUS FORMATIONS"

BY

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN MICROBIOLOGY

FACULTY OF SCIENCE
DEPARTMENT OF MICROBIOLOGY

EDMONTON, ALBERTA
SEPTEMBER, 1964.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Penetration and Migration of Serratia marcescens in Petroliferous Formations", submitted by Walter D. Samiroden, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science in Microbiology.

ABSTRACT

The object of the present study was to determine whether:

(1) bacteria can migrate through petroliferous formations found in Alberta.

(2) there is any correlation between the permeability of the formation and the rate of migration.

(3) the presence of oil in the formation has any effect on bacterial migration.

Oil was extracted from the various cores using carbon tetrachloride as the solvent in a Soxhlet apparatus. Completion of the extraction was determined by spectrophotometric methods.

Serratia marcescens, used as the test organism, was labelled with radiophosphorus and migration through the various cores was studied. The loss of the P^{32} from the labelled cells during the migrations period is shown to be low. After migration had proceeded for 48 hours, the cores were split along the longitudinal axis. A replica of the viable test organisms on the split surface was made by pressing the split surface into nutrient agar and subsequent incubation of the agar plate. Detection of accumulated labelled test organisms along the split surface was made by Geiger-Muller tube readings and autoradiography.

The test organism was found to migrate through the various petroliferous formations whether oil was present or not and without correlation to permeability or per cent effective porosity.

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to:

Dr. G.E. Myers, supervisor of the project, for his guidance, assistance and encouragement during the preparation of this thesis.

Dr. J.N. Campbell and Dr. T. Yamamoto for advice and encouragement on various aspects of these studies.

Dr. D.L. Flock, Department of Chemical and Petroleum Engineering, for information on properties of petroliferous formations and methods of measuring these properties.

Mr. D.R. Shaw, Alberta Oil and Gas Conservation Board, for his aid in having various analyses done, supplying samples of crude oil from each petroliferous formation studied and information on properties of petroliferous formations.

Core Laboratories-Canada, Limited; Edmonton, Alberta, for their measurements of the core permeabilities and effective porosities.

Bradford Laboratories, A Division of Calgon Corporation, (Canada), Limited, Edmonton, Alberta, for valuable analyses of expandable clay content in cores from the different petroliferous formations.

Mrs. N. Samiroden for the excellent typing of this manuscript.

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INTRODUCTION

Numerous reports have been published on the penetration and migration of bacteria under increased pressure through porous systems (see Woodhead and Wood, 1894, 1898; Warren and Mudd, 1924; Merkt, 1943; Plummer *et al.*, 1944; Plummer and Walling, 1946; Fekete, 1959; and Raleigh, 1962). Recently Myers and McCready (1964b) studied the penetration and migration of *Serratia marcescens* under atmospheric pressure through Berea sandstone.

Numerous species of viable bacteria have been found in various petroliferous formations, (see Zobell, 1952, 1959). Some of these bacteria can be advantageous to the petroleum industry, (see Zobell, 1947 a, b; and Robinson, 1963), others can be disadvantageous (see Merkt, 1943; Zobell, 1946, 1959; Lada, 1959; Foster, 1962; Ashirov and Sazonova, 1962; and Myers and Slabyj, 1962). Water injection is used to a great extent during secondary oil recovery.* The waters used for this operation have been found to be highly contaminated with various micro-organisms, (see Myers and Slabyj, 1962) which can plug the formations, (see Merkt, 1943; Beck, 1946, 1947; Lada, 1959; and Fekete, 1959). These data have provoked the present study to determine whether bacteria could migrate through oil-containing and oil-extracted cores taken from various petroliferous formations of Alberta.

A modification of the technique, developed by Myers and McCready (1964b), for studying penetration and migration of a motile organism through porous material was used in the present study.

*See "Monthly Statistics" Alberta Oil and Gas Industry, March 1964. Oil & Gas Conservation Board, Calgary, Alberta.

This thesis is divided into two main parts; Part I deals with the development of a method for labelling *Serratia marcescens* with radiophosphorus which would supply cells which have a significant label for tracing and yet do not leach enough label to affect the results. Part II deals with the studies of penetration and migration of the labelled organism through the various cores.

PART I

EXPERIMENTAL

The organism used in these studies was _Serratia marcescens_, Bizio ATCC (American Type Culture Collection) #272 or NCTC (National Collection of Type Cultures) #1377.

The following experiments were designed to investigate various factors involved in labelling _Serratia marcescens_ with radiophosphorus. The objective was to culture the test organism in liquid medium containing a concentration of radiophosphorus high enough that the labelled cells would possess sufficient radioactivity to facilitate tracing of migration in cores of petroliferous formations. At the same time, the concentration must be low enough that the integrity of the cell is not adversely effected and the amount of P^{32} leached from the labelled cells is not sufficient to give false positive results. The experiments are presented under two headings: I Growth Characteristics and Radiophosphorus (P^{32}) Assimilation by _Serratia marcescens_ and II Radiophosphorus (P^{32}) Retention by _Serratia marcescens_.

I Growth Characteristics and Radiophosphorus (P^{32}) Assimilation by _Serratia marcescens_

Growth Characteristics

Three methods were employed in these studies:

(i) Bacto nutrient broth containing 0.5% w/v glucose was dispensed in 150 ml volumes in 500 ml flasks. Each flask was inoculated with 0.3 ml of an 8 hour broth culture of _Serratia marcescens_. The flasks were incubated in the constant temperature bath of the Warburg apparatus at 30°C for 13 hours. Special aluminum flask holders (similar to those

used by Runyan and Geyer, 1963) which would attach to the monometer fittings of the Warburg apparatus, were designed for these experiments. At one hour intervals, 0.2 ml of the growing culture was removed and serially diluted; 0.1 ml of each dilution was then plated out on nutrient agar plates to determine the number of viable cells.

(ii) Flasks containing inoculated medium as described above were agitated in an Eberbach water bath shaker at 120 cycles/min. at 30°C for 13 hours. Viable cell counts were done as described.

(iii) The same amount of medium and inoculum was used for culturing in stationary Roux bottles at 30°C for 13 hours and viable counts were done as described. The results of these experiments are presented in Table I and illustrated graphically in Figure 1.

TABLE I

VIABLE CELL COUNT OF SERRATIA MARCESCENS

(A.T.C.C. #272) (INCUBATED AT 30°C)

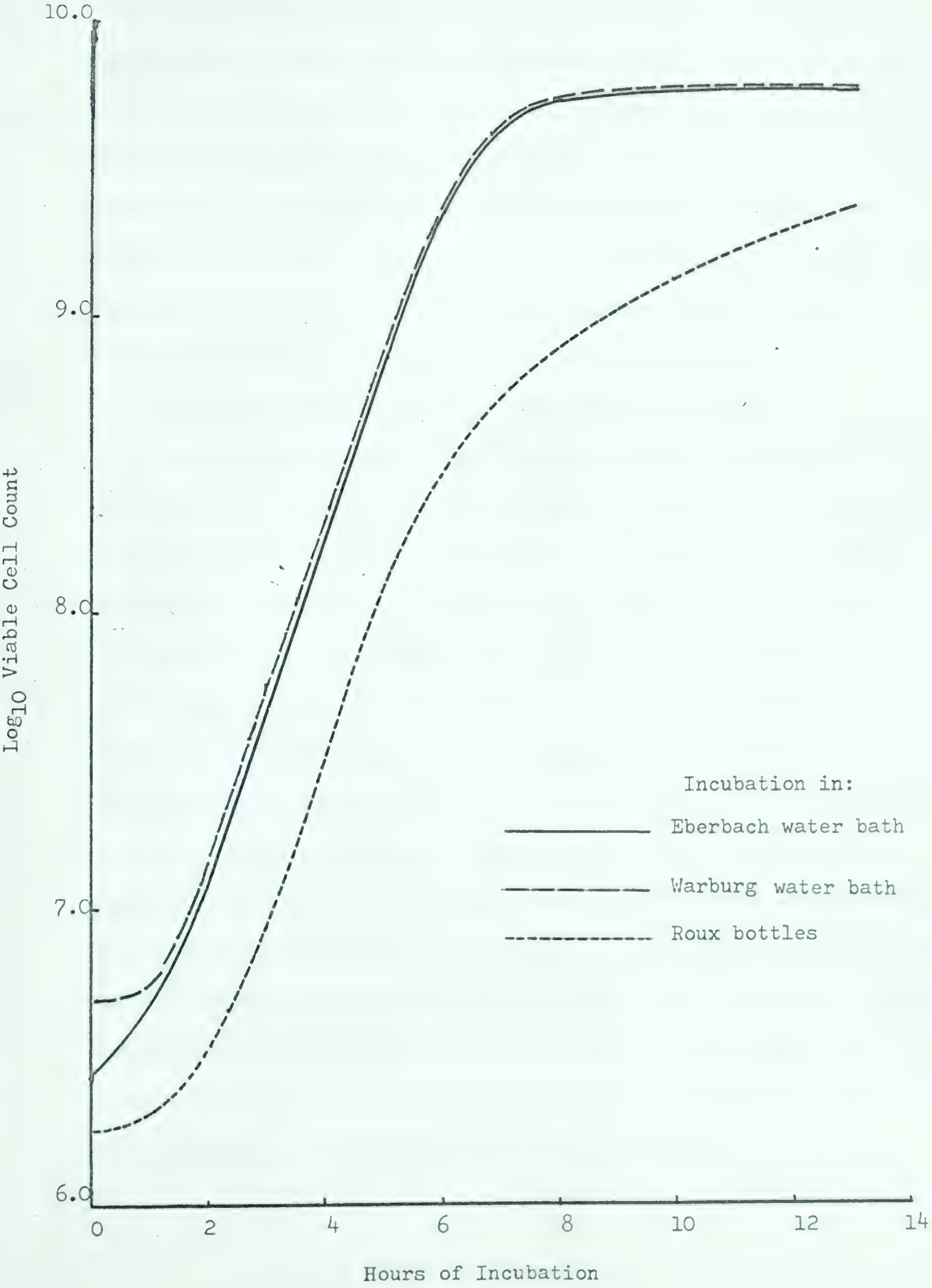
GROWN IN:

Duration of Incubation (Hours)	Warburg Water Bath (cells/ml)	Controlled Temperature Water Bath Shaker (cells/ml)	Roux Bottle (cells/ml)
0	4.9×10^6	2.8×10^6	1.7×10^6
1	5.3×10^6	4.9×10^6	2.0×10^6
2	1.7×10^7	8.8×10^6	3.4×10^6
3	5.8×10^7	4.7×10^7	1.0×10^7
4	2.2×10^8	2.1×10^8	3.6×10^7
5	7.5×10^8	7.6×10^8	2.2×10^8
6	2.7×10^9	2.7×10^9	3.4×10^8
7	4.9×10^9	4.7×10^9	5.0×10^8
8	5.8×10^9	5.7×10^9	8.1×10^8
9	6.1×10^9	6.0×10^9	7.4×10^8
10	6.2×10^9	6.1×10^9	1.3×10^9
11	6.2×10^9	6.1×10^9	1.5×10^9
12	6.3×10^9	6.3×10^9	1.9×10^9
13	6.3×10^9	6.2×10^9	2.8×10^9

FIGURE I

VIABLE CELL COUNT OF *SERRATIA MARCESCENS* (ATCC # 272)

(INCUBATED AT 30°C)



From the results presented in Table I and illustrated in Figure 1, it can be seen that the growth in the mechanically aerated cultures follows the classic growth curve. The time required for *Serratia marcescens* to reach the stationary growth phase is approximately eight hours and the maximum concentration of cells is the same for both methods. The growth of *Serratia marcescens* in Roux bottles on the other hand is much slower. The logarithmic growth phase begins to decrease after eight hours of incubation. The viable cell count continues to increase for approximately 13 hours and the maximum concentration of cells is less than was obtained in the mechanically aerated cultures.

Radioactive Phosphorus (P^{32}) Assimilation Studies

Radioactive phosphorus ($H_3P^{32}O_4$ in dilute HCl) obtained from the Atomic Energy of Canada Limited, Chalk River, Ontario, was added to a known volume of the medium. Three one ml aliquots were removed and dried on planchets and counted to determine the amount of radioactivity available in the medium for cell assimilation. The medium was then inoculated with cells from an eight hour broth culture. The cultures were incubated at 30°C for eight hours in the constant temperature water bath shaker, or, when Roux bottles were used, in the 30°C incubator. After incubation, viability counts were done as previously described. Simultaneously, the cells were harvested using a Servall refrigerated centrifuge at 4°C at 10,000 rpm for ten minutes, resuspended in distilled water and washed three successive times to remove any loosely bound phosphorus. The P^{32} assimilation was calculated by resuspending the washed cell pellet in a known volume of distilled water and counting the radioactivity of three one ml aliquots of the suspension dried on planchets.

The counts were determined by using a Nuclear Chicago Model 186 A Decade Scaler at an operating voltage of 550 volts and a sensitivity setting of 250 with a Model D-29 Geiger-Muller Tube which has a 1.4 mg/cm^2 mica window. P^{32} assimilation was calculated as c/m 10^9 viable cells and also on a percentage basis by dividing the total count of radioactivity in the cells by the total radioactivity count of the original medium and multiplying by one hundred. All counts made were extrapolated to time zero by the use of the P^{32} decay factor* unless the experiment was of less than 24 hours in duration in which case all samples were saved and counted at one time.

Effect of the Concentration of P^{32} on the Viable Cell Count and the Amount of P^{32} Assimilated by *Serratia marcescens*.

This study was done in an effort to find the optimal concentration of radioactive phosphorus in the medium which would allow *Serratia marcescens* to grow and assimilate sufficient P^{32} to facilitate tracing of the labelled cells and still have a minimal lethal effect on the cells. After considering the effect of the specific activity of P^{32} on the cell viability shown by Fuerst and Stent (1956), Myers and McCready (1964a), used a concentration of 10.0 microcuries (µc) P^{32}/300 ml of media for labelling *Serratia marcescens*. Possibly a greater amount of P^{32} could be assimilated by *Serratia marcescens* without an increased lethal effect if a slightly greater concentration of P^{32} was used in the growth medium.

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* See "Radiological Health Handbook". Revised September 1960. Edited by Division of Radiological Health. Published by U. S. Dept. of Health, Education and Welfare, Washington, D.C.

Cells were grown in Roux bottles and flasks (to be incubated in the controlled temperature water bath shaker) in nutrient broth containing 0.5% w/v glucose and varying concentrations of P^{32} ranging from 0 to 12.5 µc P^{32}/150 ml of medium. The cultures were incubated for eight hours at which time viability counts were done as previously described. Simultaneously the percentages of P^{32} assimilation by the cells grown in varying concentrations of P^{32} were determined as well as the c/m 10^9 viable cells. The results are summarized in Tables II and III.

TABLE II

EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND ON THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS

(CULTURES GROWN IN ROUX BOTTLES AT 30°C)*

µc P^{32} per 150 ml media	Radio-activity of media c/m	Radio-activity of cells c/m	Per cent P^{32} assimilation	Viable cell count/ml X 10^{-9}	Radio-activity of cells c/m 10^9 cells
0	0	0	0	4.80	0
5.0	441,600	102,600	23.23	4.85	141.03
7.5	673,350	149,400	22.19	4.83	206.21
10.0	883,350	200,100	22.65	4.74	281.58
12.5	1,185,600	252,600	21.31	4.77	353.14

* Results are tabulated in detail in Appendix B, Table I.

TABLE III

EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND ON THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS

(CULTURES GROWN IN 500 ML. FLASKS AT 30°C IN EBERBACH WATER BATH SHAKER)*

μc P^{32} per 150 ml media	Radio-activity of media c/m	Radio-activity of cells c/m	Per cent P^{32} assimilation	Viable cell count/ml X 10^{-9}	Radio-activity of cells c/m 10^9 cells
0	0	0	0	8.6	0
5.0	588,600	243,947	41.44	8.75	185.88
7.5	916,800	375,632	40.97	8.87	242.32
10.5	1,150,500	485,684	42.21	8.5	280.92
12.5	1,522,950	586,579	38.51	8.4	465.46

* Results are tabulated in detail in Appendix B, Table II.

The effect of aeration on the growth of *Serratia marcescens* is again seen in the results shown in Tables II and III where the viable count of the aerated cultures is almost twice that of the Roux bottle cultures. The range of P^{32} concentration used in the growth medium in this experiment does not seem to have any great effect on the respective viable counts. The per cent P^{32} assimilated by the aerated culture is also almost twice the per cent P^{32} assimilated by the Roux bottle grown cells, however, as shown in the last column in Table II and III, there is no great increase in the radioactivity of the individual cell. *Serratia marcescens* assimilates approximately 40% of the available P^{32} in the growth medium when grown in the Eberbach water bath shaker at 120 cycles/minute at 30°C for 8 hours.

Effect of the Concentration of Glucose on the Viable Cell Count and the Amount of P^{32} Assimilated.

Myers and McCready (1964a) found that the glucose concentration in the medium affected the amount of P^{32} assimilated by Serratia marcescens. A 1.0% w/v glucose concentration was more stimulatory than 1.5 and 2.0% w/v glucose concentrations. However, results using concentrations of less than 1% w/v glucose were not reported. In this study, 150 mls of nutrient broth containing 5.0 μc P^{32} and varying concentrations of glucose varying from 0 to 1.0% w/v in 500 ml flasks were inoculated with eight hour broth culture cells of Serratia marcescens and incubated at 30°C for eight hours in the Eberbach water bath shaker. Viability counts and P^{32} assimilation studies were carried out as already described. The results are given in Table IV and Figure 2.

TABLE IV

EFFECT OF THE CONCENTRATION OF GLUCOSE IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS*

(5 μc P^{32}/150 ml Media was used for the Radioactive Assay)

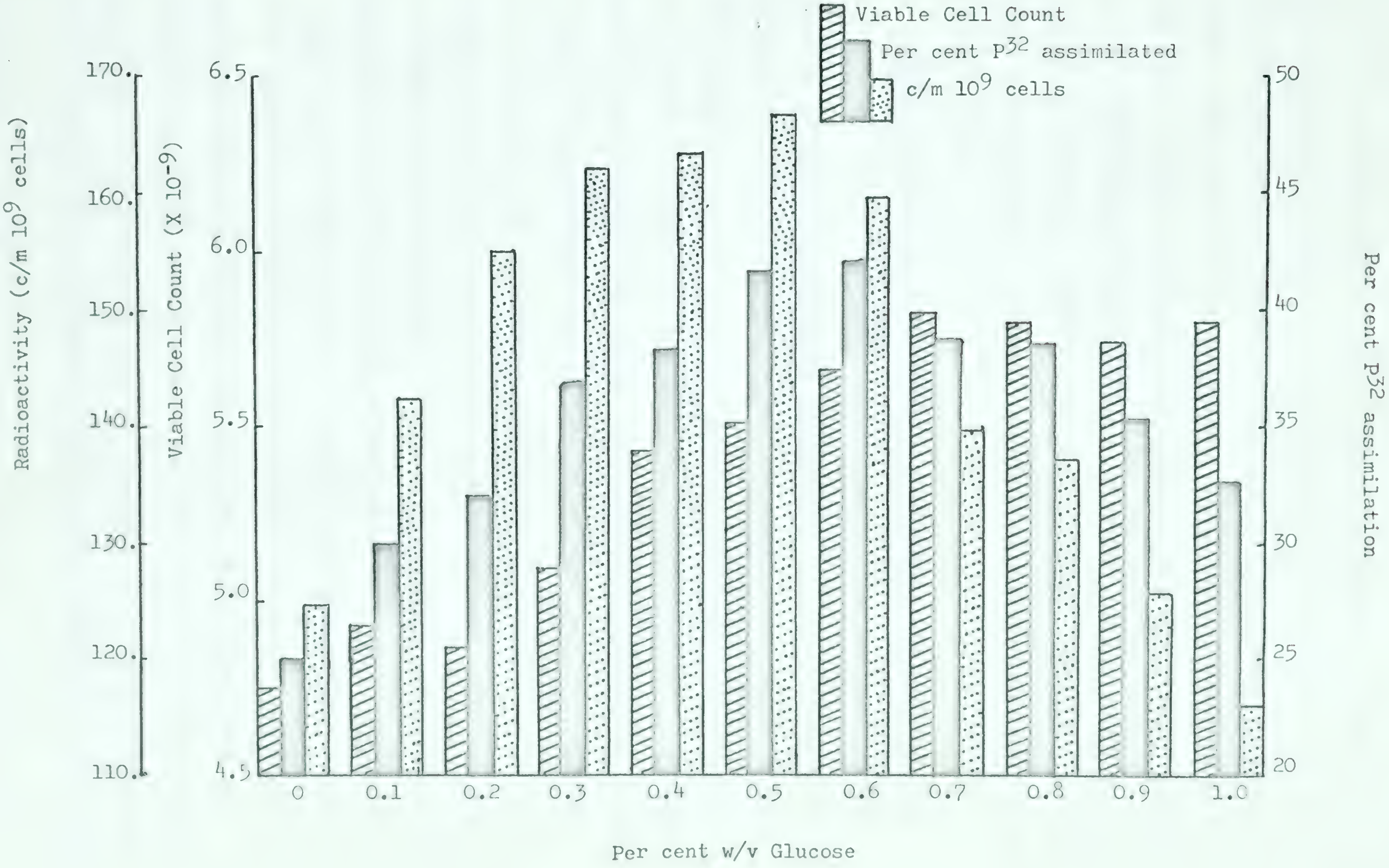
Per cent w/v Glucose	Radio-activity of media c/m	Radio-activity of cells c/m	Per cent P^{32} assimilated	Viable cell count/ml X 10^{-9}	Radio-activity of cells c/m 10^9 cells
Control	0	0	0	5.8	0
0	354,000	88,500	25.00	4.75	124.47
0.1	352,050	105,300	29.91	4.93	142.39
0.2	354,000	113,280	32.11	4.87	155.07
0.3	337,200	123,780	36.71	5.09	162.12
0.4	346,800	133,080	38.37	5.43	163.38
0.5	331,650	137,790	41.55	5.51	166.71
0.6	322,800	135,690	42.04	5.67	159.55
0.7	318,450	122,010	38.31	5.82	139.76
0.8	309,600	119,010	38.44	5.79	137.03
0.9	308,550	108,660	35.22	5.76	125.76
1.0	309,750	100,860	32.56	5.79	116.13

* Results are tabulated in detail in Appendix B, Table III.

FIGURE 2

EFFECT OF THE CONCENTRATION OF GLUCOSE IN THE GROWTH MEDIUM

ON VIABLE CELL COUNT AND P^{32} ASSIMILATION



It is evident from the results presented in Table Iv and illustrated in Figure 2, that a concentration of 0.5% w/v glucose was most stimulating toward P^{32} assimilation by Serratia marcescens. Glucose concentration also influenced the cell yield as is seen by a study of the results listed in the last two columns in Table IV. Thus, increased per cent P^{32} assimilation with increased glucose concentration does not mean that there is an increased P^{32} assimilation by the individual cell since there is also an increase in cell count with the increased glucose concentration.

Effect of Incubation Time on P^{32} Assimilation and Cell Viability.

It was found in previous experiments that the logarithmic growth phase of an aerated Serratia marcescens culture ended at approximately eight hours, (see Figure 1). It was of interest to determine whether P^{32} assimilation parallels the growth curve of the organism or occurred after cell formation. To determine this, 210 ml volumes of the nutrient broth containing 0.5% w/v glucose were dispensed in each of two 500 ml flasks. 7.0 μc P^{32} was added to one flask. Three one ml samples of the medium were removed from each flask, dried on separate planchets and the amount of P^{32} available for assimilation was determined by count. The media was then inoculated with cells from an eight hour broth culture andincubated at 30°C in the controlled temperature water bath shaker. At zero time of incubation and at each hour thereafter for 14 hours, 10 ml aliquots were removed from each culture, the cells harvested by centrifugation at 10,000 rpm for 10 minutes, washed three times and resuspended in 10 ml of distilled water. Three one ml volumes of each suspension were removed and dried on planchets for counting. The amount of P^{32} assimilated was correlated with time of incubation. Simultaneously,

titrations were done for viable cell counts. The results are presented in Table V.

TABLE V

EFFECT OF INCUBATION TIME ON VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY *SERRATIA MARCESCENS* INCUBATED IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C*

(Test Media Prior to Inoculation had a Total Count of 55,880/min)

Hours of Incubation	Radio-activity of cells c/m	Per cent P^{32} assimilated	Viable Cell Count		Radio-activity of cells c/m 10^9 cells
			Control	Test	
0	0	0	9.2×10^6	9.5×10^6	0
1	0	0	1.09×10^7	1.17×10^7	0
2	0	0	3.73×10^7	3.63×10^7	0
3	840	.15	8.73×10^7	9.76×10^7	40.98
4	2,100	.38	2.27×10^8	2.13×10^8	46.95
5	9,030	1.64	7.43×10^8	7.73×10^8	55.63
6	39,690	7.19	2.52×10^9	2.96×10^9	63.85
7	105,840	19.18	5.02×10^9	5.19×10^9	97.11
8	213,990	38.77	5.83×10^9	5.99×10^9	170.12
9	224,280	40.64	6.32×10^9	6.27×10^9	170.33
10	223,230	40.45	6.17×10^9	6.33×10^9	167.93
11	219,030	39.69	6.29×10^9	6.23×10^9	167.42
12	221,970	40.22	6.26×10^9	6.31×10^9	167.51
13	216,720	39.27	6.34×10^9	6.29×10^9	164.07
14	218,610	39.61	6.26×10^9	6.27×10^9	166.03

* Results are tabulated in detail in Appendix B, Table IV.

It can be seen from the results presented in Table V that the P^{32} assimilated by Serratia marcescens is related to its growth, P^{32} assimilation being most active during the end of the logarithmic growth phase.

II Radiophosphorus (P^{32}) Retention by Serratia marcescens.

Stability of the P^{32} Label at Room (23-25°C) and Refrigerator (4°C) Temperatures.

Myers and McCready (1964b) found water to be the best vehicle for studies of the migration of Serratia marcescens through Berea Sandstone since it allowed the least amount of P^{32} to be leached. The present studies were undertaken in order to determine the percentage of the assimilated radiophosphorus which can be found in the supernatant fraction of a suspension of labelled cells after 48 hours since the subsequent migration studies of labelled cells through petroliferous formations were to extend for this length of time. Supernatants of suspensions of the different petroliferous formations in demineralized water were used as vehicles in this study so that any effect of soluble materials from these formations on the amount of P^{32} lost from labelled Serratia marcescens would be apparent.

Samples of the various oil-bearing formations (Pekisko-limestone, Cardium-sandstone, Beaverhill Lake-limestone, Belly River-sandstone and Viking-sandstone) from which cores were prepared for migration studies of Serratia marcescens were each crushed to a fine powder by mortar and pestle. Twenty-five grams of each powder was suspended in 150 ml of demineralized water. Each suspension was mixed for 24 hours

by constant stirring on a Scientific Glass apparatus Magnestir No. S-6910. The suspension was allowed to stand till the supernatant cleared at which time 100 mls of each was removed and sterilized by autoclaving. Labelled cells (Serratia marcescens grown in nutrient broth containing 0.5% w/v glucose and 5.0 uc P^{32}/150 ml) were then suspended in each 100 ml of supernatant. To determine the amount of P^{32} leached from the cells as a function of time, 10 ml aliquots of each suspension were removed at 0 time and at 12 hour intervals thereafter for 96 hours. The cells were immediately harvested by centrifugation at 10,000 rpm for 10 minutes in the Servall RC-2 refrigerated centrifuge at 4°C and three 1 ml samples of each supernatant were removed and dried on planchets for counting. Knowing the original radioactivity of the cells in c/m, the per cent radioactivity leached from the cells during the experiment was calculated. These studies were done at room temperature (23-25°C) and under refrigeration at 4°C. The results are presented in Tables VI and VII.

TABLE VI

PER CENT P^{32} LEACHED FROM <u>SERRATIA MARCESCENS</u> AT ROOM TEMPERATURE (23-25°C)*

Cells Suspended in Supernatants of:

Hours of Suspension	Belly River	Viking	Cardium	Pekisko	Beaverhill Lake
0	0.34	0.41	0.81	0.72	0.96
12	3.36	3.04	4.97	3.95	4.47
24	5.68	5.45	6.86	5.39	5.72
36	7.42	7.39	7.73	8.17	7.53
48	9.19	9.07	10.85	10.68	9.01
60	12.09	11.45	12.87	12.63	11.99
72	16.84	15.89	16.37	16.60	15.72
84	19.25	18.79	18.62	20.14	19.3[illegible]
96	21.65	22.20	20.70	22.40	21.3[illegible]

*Results are tabulated in detail in Appendix B, Table V.

TABLE VII

PER CENT P^{32} LEACHED FROM <u>SERRATIA MARCESCENS</u> AT REFRIGERATOR TEMPERATURE (4°C)*

Cells Suspended in Supernatants of:

Hours of Suspension	Belly River	Viking	Cardium	Pekisko	Beaverhill Lake
0	1.00	0.93	0.87	0.89	0.87
12	4.51	5.02	4.00	3.94	3.97
24	4.76	5.37	4.82	4.49	4.61
36	6.39	7.47	5.05	4.77	5.02
48	7.27	7.47	6.56	6.32	6.48
60	8.77	8.52	7.73	7.52	7.71
72	8.39	9.74	8.94	8.42	8.86
84	9.77	11.02	10.08	9.70	10.08
96	9.91	11.27	10.69	10.28	10.62

*,Results are tabulated in detail in Appendix B, Table VI.

It can be seen from a comparison of the results given in Tables VI and VII that during the 96 hour duration of the experiment, a significant increase (average of 11.16%) occurs in the amount of P^{32} leached at room temperature (23-25°C) over the amount at refrigerator temperature (4°C). From Table VII, it is predicted that approximately 8% of the label could leach from the labelled cells in suspension during migration studies carried out at 4°C for 48 hours.

The Effect of the Concentration of P^{32} in the Growth Medium on P^{32} Retention and Viability of *Serratia marcescens* in Demineralized Distilled Water Suspension at 4°C.

P^{32} labelled cells were to be used subsequently for migration studies. Therefore, it was necessary to determine the effect of the P^{32} concentration in the growth medium on the viability and leaching rate of P^{32} from the water suspended cells. In the previous experiment (Tables VI and VII), it is shown that a lower percentage of the assimilated P^{32} is lost from labelled *Serratia marcescens* cells when they are stored at 4°C then when they are stored at 23-25°C. Also from preliminary studies, it is obvious that the cells assimilate a constant percentage of the available P^{32} when grown under conditions such that the cell yield is constant. Thus, cells grown in a medium of high specific activity* necessarily assimilate a greater amount of P^{32} than the cells grown in a medium of low specific activity. For migration studies, it is preferable to use organisms grown in a high specific activity medium since

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* Specific Activity (S/A) = $\mu g. P^{32}/\mu g.$ total phosphorus.

they possess greater radioactivity. However, the obvious questions as to the stability of the label in the cells and the effect of the increased label on the mortality of cells remain to be answered.

In the present experiment, nutrient broth was used containing 0.5% w/v glucose and various concentrations of P^{32} ranging from 0 to 20.0 µc/75 ml. The medium was inoculated and incubated as previously described. The cells were harvested, washed, resuspended in 75 ml of demineralized distilled water and stored at 4°C for 72 hours. At 0 time and at 12 hour intervals thereafter, viable counts were made and the per cent P^{32} lost from the cells determined. The results are given in Table VIII and illustrated graphically in Figure 3.

TABLE VII

EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE P^{32} RETENTION AND THE VIABLE CELL COUNT OF LABELLED <u>SERRATIA</u> <u>MARCESCENS</u> IN DEMINERALIZED DISTILLED WATER AT 4°C *

	Cells Suspended for:							
	0 Hours		12 Hours		24 Hours		36 Hours	
P^{32} in media μc/75 ml	Per cent P^{32} assimilated	Viable cell count/ml X 10^{-9}	Per cent P^{32} leached	Viable cell count/ml X 10^{-9}	Per cent P^{32} leached	Viable cell count/ml X 10^{-9}	Per cent P^{32} leached	Viable cell count/ml X 10^{-9}
0	0	6.03	0	4.75	0	3.05	0	2.43
2.5	38.36	6.12	3.62	4.71	4.27	3.12	5.14	2.24
3.75	40.03	6.21	3.31	4.51	4.81	3.09	4.97	2.35
5.0	37.89	6.17	3.07	4.32	4.44	2.91	5.15	2.06
6.25	37.86	6.15	3.51	4.21	4.10	3.04	5.03	2.03
7.5	37.89	6.09	3.61	3.71	4.73	2.58	5.79	1.67
10.0	37.63	6.03	3.93	3.64	4.73	2.12	5.50	1.24
15.0	37.87	5.91	3.64	3.32	3.79	2.03	5.81	1.08
20.0	37.86	5.87	4.03	3.51	5.05	1.92	6.36	1.14

* Results are tabulated in detail in Appendix B, Table VII

TABLE VIII CONTINUED

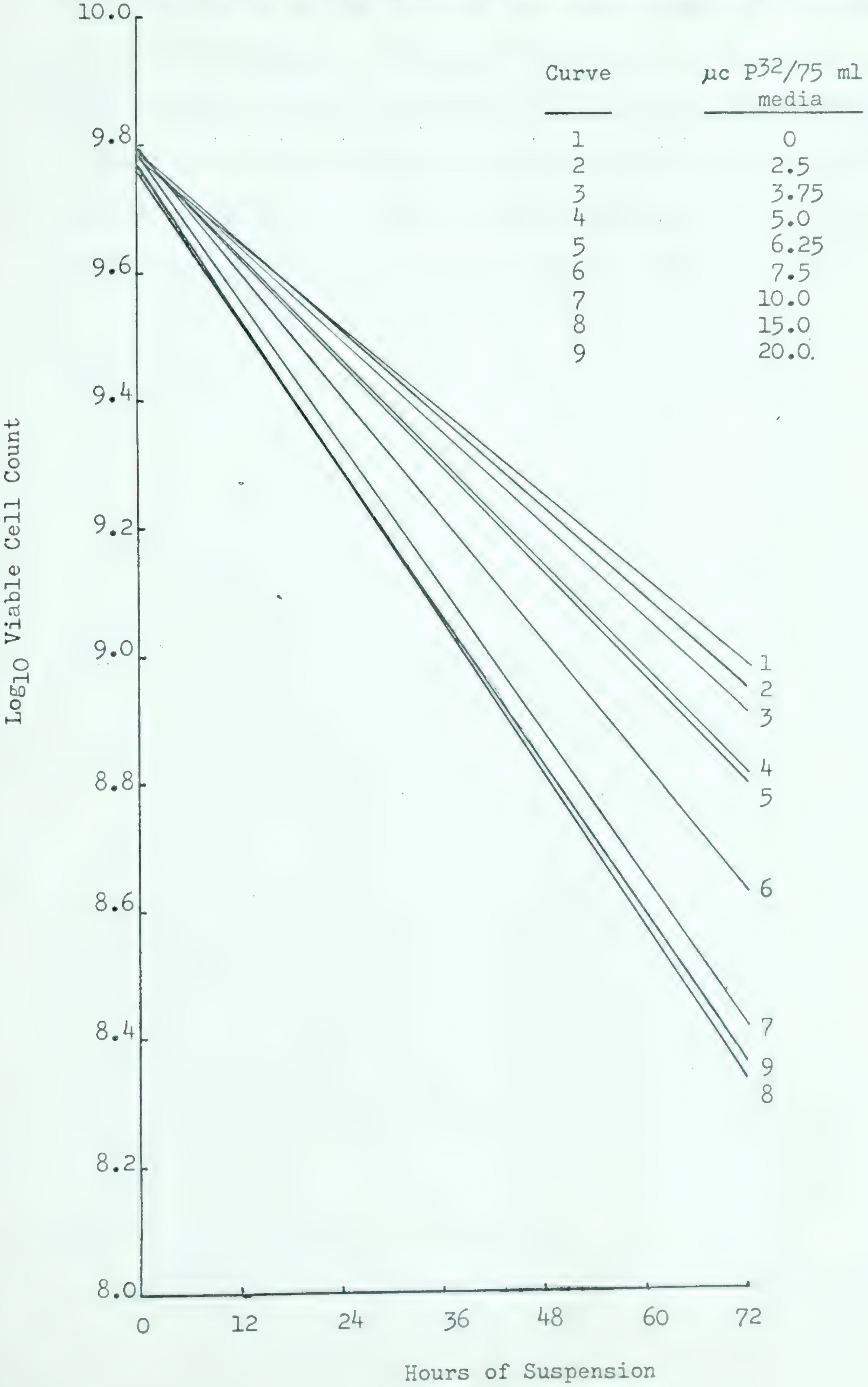
EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE P^{32} RETENTION AND THE VIABLE CELL COUNT OF LABELLED *SERRATIA MARCESCENS* IN DEMINERALIZED DISTILLED WATER AT 4°C

Cells Suspended for:

	48 Hours		60 Hours		72 Hours	
P^{32} in media µc/75 ml	Per cent P^{32} leached	Viable cell count/ml	Per cent P^{32} leached	Viable cell count/ml	Per cent P^{32} leached	Viable cell count/ml $X 10^{-8}$
0	0	$1.68 X 10^9$	0	$1.35 X 10^9$	0	9.2
2.5	5.42	$1.63 X 10^9$	5.96	$1.31 X 10^9$	6.25	8.7
3.75	5.24	$1.65 X 10^9$	5.77	$1.10 X 10^9$	6.15	8.4
5.0	5.36	$1.29 X 10^9$	5.85	$1.05 X 10^9$	6.07	6.3
6.25	5.31	$1.33 X 10^9$	5.80	$1.04 X 10^9$	6.04	6.5
7.5	5.97	$9.6 X 10^8$	6.41	$6.6 X 10^8$	6.66	4.4
10.0	5.79	$7.3 X 10^8$	6.31	$4.7 X 10^8$	6.62	2.71
15.0	6.10	$6.8 X 10^8$	6.61	$3.8 X 10^8$	6.92	2.04
20.0	6.54	$7.1 X 10^8$	7.04	$4.1 X 10^8$	7.33	2.15

FIGURE 3

EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT OF THE LABELLED CELL SUSPENSION AS A FUNCTION OF TIME



It can be seen from a study of the results shown in Table VIII that the per cent of P^{32} leached from labelled Serratia marcescens increases as the concentration of P^{32} in the growth medium increases. Also the results given in Table VIII and Figure 3 show that cells grown in media with P^{32} concentration of 2.5 and 3.75 μc P^{32}/75 ml of media have a mortality rate comparable with the mortality rate of cells grown in the absence of radiophosphorus. Cells grown in a media of high P^{32} concentration have a higher mortality rate.

DISCUSSION

Growth Characteristics of Serratia marcescens.

The increase in the viable cell count of Serratia marcescens was studied as a function of time by the method of plating serial dilutions. This method has a number of inherent sources of error, however, since only a comparative study was intended, it was felt that the method used was adequate. Some technical error was eliminated in the preparation of the 1.8 ml water blanks used for the dilutions. In preliminary studies, it was found that approximately 0.25 ml of the water blanks evaporated during sterilization by autoclaving. Evaporation also continued during storage at 4°C. Thus, in the plate counts made from plating out 0.1 ml of each dilution, one would arrive at a higher viable cell count than would be found if all the blanks contained exactly 1.8 ml of water at the time of dilution. This error was eliminated by aseptically dispensing the 1.8 ml volumes of water into the sterile dilution tubes after, rather than before, autoclaving.

As seen from Figure 1, Serratia marcescens growth followed the classic growth curve for bacteria, (see Salle, 1961). The difference in the curve for growth in the Roux bottles as compared to that obtained with shaking is likely due to the difference in the degree of aeration, (see Smith and Johnson, 1954). Aeration of aerobic bacterial cultures has a twofold effect. Firstly, there is the Pasteur Effect seen with microorganisms such as Serratia marcescens. Secondly, there is the physical effect brought about by the agitation which reduces clumping of the organism.

The growth curves obtained by incubation of Serratia marcescens

in the Warburg apparatus and the Eberbach water bath shaker were comparable. Only the Eberbach water bath shaker was employed in subsequent experiments.

Cells which have just reached the end of the logarithmic growth phase would be the most suitable for tracer studies since they have the lowest mortality rate (see Salle, 1961) and also have likely assimilated the maximal amount of P^{32} (see McCready, 1963). Since the logarithmic growth phase ends at approximately 8 hours of incubation (see Figure 1), in subsequent experiments, cells were harvested at this time, (see Part II), except when radiophosphorus assimilation was studied as a function of time. Incubation was then allowed to proceed for 14 hours.

Effect of the Concentration of P^{32} in the Growth Medium on the Viable Cell Count and the Amount of P^{32} Assimilated.

Earlier studies showed that bacterial cultures either did not grow in medium containing a very high concentration of radiophosphorus, (Fuerst and Stent, 1956, and Labaw *et al.*, 1950), or there was a great loss of viability believed to result from disintegrations occurring in the bacterial DNA strands, (see McFall *et al.*, 1958). Myers and McCready, (1964a) found that the assimilated radiophosphorus remained at constant levels in *Serratia marcescens* with the label being distributed in the riboncleic acid, acid soluble, deoxyribonucleic acid and lipid fractions in decreasing quantities. Fuerst and Stent, (1956) suggested that the decrease in *E. coli* multiplication resulted from decay of P^{32} atoms which had been assimilated into the cells and not from the radiation emitted by the extracellular P^{32} . They found that when an intermediate specific activity of radio-

phosphorus was used in the growth medium, cells which were slightly filamentous were produced. Since, in our experiments, there was neither a significant decrease in the viability nor any change in the bacterial morphology of *Serratia marcescens* grown in medium containing concentrations of P^{32} between 0 and 12.5 µc/150 ml., such cells should be suitable for tracer studies.

Effect of the Concentration of Glucose in the Growth Medium on the Viable Cell Count and the Amount of P^{32} Assimilated.

In preliminary studies, it was found that changes in the concentration of glucose affected both the P^{32} assimilation and the viable cell count. Myers and McCready (1964a) state that 1% w/v glucose in the culture medium stimulates phosphorus assimilation. However, in the present study where radiophosphorus assimilation is correlated with the viable cell count for concentrations of glucose ranging from 0 to 1.0% w/v in the medium (Table IV), the increase in viable count with increasing glucose concentrations is greater than the corresponding increase in P^{32} assimilation. *Thus, the optimal concentration of glucose for P^{32} assimilation is 0.5% w/v* and actually, 10^9 viable cells grown in 1.0% w/v glucose have a lower level of radioactivity than 10^9 viable cells grown in the control (without added glucose) medium.

Increased viable cell count of bacteria with increasing glucose concentrations has been seen with a number of organisms. DeMoss *et al.*, (1951) and Sokatch and Gunsalus, (1957) showed a linear relationship between the dry weight of the organism grown and the glucose concentration of the medium with both *Streptococcus faecalis* and *Leuconostoc mesenteroides*. Bauchop and Elsden, (1960) show this same phenomenon

to be true with *Pseudomonas lindneri*, *Saccharomyces cerevisiae* and *Propionibacterium pentosaceum*. With *Serratia marcescens*, where there is also an increased yield with increased glucose concentrations, it is possible that there is a greater initial P^{32} assimilation in the form of ATP via oxidative phosphorylation, (see Bauchop and Elsden, 1960, and Niedhardt and Magasanik, 1956).

Radiophosphorus (P^{32}) Retention by *Serratia marcescens*.

Using low concentration of P^{32} in the growth medium, Labaw *et al*., 1950 and Caldwell *et al*., 1950, showed that very little P^{32} is lost from the growing cell, however, in our work with cell suspensions, (see Table VIII), there is a higher rate of P^{32} loss by leaching with increased assimilation of $P^{32}/10^9$ viable cells.

The generally accepted pathway for phosphate assimilation, (see Kamen and Speigelman, 1948; Labaw *et al*., 1950; Caldwell *et al*., 1950; Mitchell and Moyle, 1953; and Rothstein, 1959), is that the phosphate enters the cell by an esterification process at the cell membrane followed by mixing into the metabolic pool. From the metabolic pool, the phosphate is incorporated into metabolic pathway intermediates and is finally assimilated into the nucleic acids being more stable in the deoxyribonucleic acid fraction, (see Labaw *et al*., 1950). Thus it is agreed that the P^{32} lost from the cells is mainly from the metabolic pool prior to incorporation into the nucleic acid fraction if the cells are grown in medium of low specific activity. On the other hand, if cells are grown in medium of high specific activity, the P^{32} incorporated into the nucleic acid fraction may also be lost as a result of the disruption of DNA strands due to the breakage of ester links, (see Stent and Fuerst, 1955 and McFall

et al., 1958)

It is also possible that glucose concentrations may affect the amount of leaching of the assimilated P^{32} from *Serratia marcescens* as it does with *Staphylococcus aureus* and yeast. Rothstein, (1959), points out that when glucose is present, the phosphate moves into the cell at the same rate but almost none moves out.

From our studies of leaching with *Serratia marcescens*, the single factor which has the greatest effect is the temperature, there being a lower loss of P^{32} at a lower temperature. Thus, subsequent migration studies (see Part II), were done at 4°C.

Effect of the Concentration of P^{32} in the Growth Medium on the P^{32} Retention and the Viable Cell Count of *Serratia marcescens* in Demineralized Distilled Water Suspensions at 4°C.

Earlier we have shown, (with regard to lethal effect) that P^{32} concentrations ranging from 0 to 12.5 µc/150 ml of medium should be satisfactory for producing labelled cells intended for use in subsequent migration studies, (see Tables II and III). From the present studies, (see Table VIII), it is evident that leaching of P^{32} from the labelled cells grown in medium containing 2.5 to 6.25 µc P^{32}/75 ml is constant, and that the mortality rate is constant for cells grown in medium containing 2.5 and 3.75 µc P^{32}/75 ml (see Table VIII and Figure 3). For these reasons, a concentration of 7.5 µc P^{32}/150 ml of medium was used for labelling Serratia marcescens for subsequent migration studies.

CONCLUSIONS

Subsequent to the results obtained in the foregoing preliminary studies on P^{32} assimilation by Serratia marcescens, cells to be used in migration studies were grown in nutrient broth containing 0.5% w/v glucose and 7.5 µc P^{32}/150 ml and incubated in the Eberbach water bath shaker operated at 120 cycles/minute at 30°C for 8 hours.

PART II

EXPERIMENTAL

PENETRATION AND MIGRATION OF P^{32} LABELLED <u>SERRATIA</u> <u>MARCESCENS</u> IN CORES TAKEN FROM VARIOUS PETROLIFEROUS FORMATIONS OF ALBERTA

MATERIALS AND METHODS

The cores* used in our studies were one inch in diameter, three inches in length and cut parallel to the bedding plane. Various characteristics of those cores are given in Table II.

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* All cores were supplied by the Oil and Gas Conservation Board, Calgary, Alberta.

TABLE IX

CHARACTERISTICS OF THE CORES FROM VARIOUS PETROLIFEROUS FORMATIONS OF ALBERTA

1 Twinning Field, Pekisko Formation (Limestone)

Core No.	Permeability* md.	Per cent** effective Pore Space	Oil Extraction	Duration of *** Extraction Hours
1	less than 0.1		+	384
2	less than 0.1	4.2	-	0
3	0.5		+	336
4	0.6		-	0
5	34.0		+	288
6	41.0	10.1	-	0
7	13.0		+	360
8	28.0		-	0
9	13.0		-	0
10	15.0		-	0
11	9.5		+	408
12	9.9		-	0
13	7.2		+	384
14	0.5		-	0
15	6.6		-	0

* Permeability - A porous medium has a permeability of one darcy when a single phase fluid of one centipoise viscosity that completely fills the voids of the medium will flow through it under conditions of viscous flow at a rate of one cubic centimeter/second sq. cm. cross sectional area under a pressure of one atmosphere/sq. cm.

** Per cent effective Pore Space - Per cent volume of a medium made up of communicating pores available to the atmospher.

*** See Appendix C.

Permeability and Per cent effective Pore Space measurements were done by Core Laboratories-Canada Ltd., Edmonton, Alberta.

II Pembina Field, Cardium Formation (Sandstone)

Core No.	Permeability md.	Per cent effective Pore Space	Oil Extraction	Duration of Extraction Hours
1	2.5		+	168
2	1.0	12.8	-	0
3	4.6		-	0
4	6.7	15.1	+	192

III Swan Hills Field, Beaverhill Lake Formation (Limestone)

Core No.	Permeability md.	Per cent effective Pore Space	Oil Extraction	Duration of Extraction Hours
1	1.7		-	0
2	less than 0.1	3.3	+	192
3	0.5		-	0
4	36.0		+	216
5	30.0		-	0
6	306.0	16.9	-	0

IV Willesden Green Field, Belly River Formation (Sandstone)

Core No.	Permeability md.	Per cent effective Pore Space	Oil Extraction	Duration of Extraction Hours
1	6.6		-	0
2	9.7		-	0
3	less than 0.1	11.2	-	0
4	28.0	20.9	-	0
5	0.8		+	168

V Beaverhill Lake Field, Viking Formation (Sandstone)

Core No.	Permeability md.	Per cent effective Pore Space	Oil Extraction	Duration of Extraction Hours
1	283.0	19.2	-	0
2	168.0		+	168
3	143.0		-	0
4	118.0		-	0
5	3.2	20.1	-	0

Oil Extractions

The cores in each group were paired according to permeability measurements and the oil was then extracted from one core of each pair. Thus, comparisons would be made of the migrations of labelled *Serratia marcescens* through extracted and non-extracted cores of similar permeabilities from the same formation. Oil extraction was done in a glass Soxhlet apparatus using technical grade carbon tetrachloride as the solvent. Each core was subjected to a series of 24 hour extractions. Depending on the size of the Soxhlet apparatus used, from 150 ml to 250 ml of solvent was used during each 24 hour period of extraction. A check on the degree of extraction was done spectrophotometrically using a modification of the method described by Hillis, (1937). Crude oil* from each formation was diluted in carbon tetrachloride to a concentration which could be scanned on a D.U. Spectrophotometer. Each crude oil exhibited maximal absorption at a characteristic wave length. At the end of each 24 hour extraction period, the solvent was removed from the apparatus and the optical density was measured, (at the wave length of maximal absorption as previously determined for the particular crude oil) and compared with the optical density of carbon tetrachloride alone. Fresh solvent was used for each 24 hour extraction. When no measureable absorption by the solvent in excess of the control could be detected, the extraction was assumed to be complete.** The cores were then removed and dried in a vacuum of 770 mm. of mercury for 24 hours

* Crude oil samples were obtained from the Oil and Gas Conservation Board, University of Alberta, Edmonton.

** Optical density measurements for each 24 hour extraction sample of the different petroliferous formation cores extracted are given in Appendix C.

at room temperature (23-25°C).

Toxic Effects of Extracted and Non-Extracted Core Material on *Serratia marcescens*.

Since migration of the labelled viable bacteria was to be compared through both oil-extracted and non-extracted cores, it was necessary to determine whether the oil-extracted or non-extracted core material had any toxic effect on the bacteria. For this study, the bacteria were grown in nutrient broth containing 0.5% w/v glucose and 7.5 μc P^{32}/150 ml. The labelled cells were harvested, washed three times and resuspended in demineralized distilled water. Twelve such suspensions were prepared. To each of five suspensions was added 25 grams of ground core material from one of the five formations being studied. To each of five of the remaining suspensions, 25 grams of ground oil-extracted material from the same formations were added. One suspension was left as a control without any added core material and to the remaining suspension was added 25 grams of washed ground core material from a non-petroliferous sandstone (Berea). A viable cell count was done on the suspension in each flask at 0 hours and after a 48 hour period of storage at 4°C. The decrease in the viable count over the 48 hour period is comparable for all the suspensions tested (see Table X). The decrease in the viable cell count for all the suspensions is also comparable with that found in previous experiments when 7.5 μc P^{32}/150 ml of medium was used (See Table VIII, Part I). There does not seem to be any great difference between the decrease in the viable count in the non-extracted petroliferous samples and the decrease in the viable count in the extracted petroliferous samples over the 48 hour period.

TABLE X

TOXIC EFFECTS OF EXTRACTED AND NON-EXTRACTED CORE MATERIAL

ON SERRATIA MARCESCENS

Ground Material Added to Water	Viable Count In: Oil-Extracted Material 0 Hours	Oil-Extracted Material 48 Hours	Non-Extracted Material 0 Hours	Non-Extracted Material 48 Hours
0			7.23×10^9	5.18×10^9
Non-petroliferous sandstone			7.47×10^9	5.10×10^9
Twinning Field, Pekisko formation	7.15×10^9	5.16×10^9	7.03×10^9	5.02×10^9
Swan Hills Field, Beaverhill Lake formation	7.18×10^9	4.94×10^9	7.41×10^9	5.16×10^9
Willesden Green Field, Belly River formation	7.19×10^9	4.97×10^9	7.27×10^9	5.14×10^9
Beaverhill Lake Field, Viking formation	7.36×10^9	5.17×10^9	7.12×10^9	5.06×10^9
Pembina Field, Cardium formation	7.24×10^9	5.23×10^9	7.19×10^9	5.27×10^9

Core Preparation

The external perimeter of each core was sealed with epoxy resin, (see Myers and McCready, 1964b), to prevent evaporation of fluid from the surface during the experiments. Three portals, 1/4 inch in diameter, were drilled through the epoxy coating to the core surface along four longitudinal axes of the core at 90° intervals around the circumference. The first portal along each axis was drilled 1 1/4 inches from the end of the core at which the bacteria would enter and the others were drilled at 3/4 inch intervals.

The prepared cores were then subjected to a reduced pressure of 770 mm. of mercury for 24 hours at room temperature (23-25°C). Subsequently, the vacuum was broken to allow the evacuated core to equilibrate at atmospheric pressure. During the core equilibration, cells were grown, harvested and washed as previously described, (see Part I). The washed cell pellet was resuspended in sterile demineralized distilled water to give a final volume of 130 ml. A sample was taken for plate count determination and three 1 ml samples were dried on planchets for P^{32} assimilation determination. A measured volume, (65-75 ml), of the labelled cell suspension was transferred to the lucite core holder, (see Myers and McCready, 1964b). The prepared core was removed from the dessicator and placed in the lucite core holder. Suspension of the cells was maintained by bubbling air through the slurry during the migration experiment. The lucite core holder, viable labelled cell suspension and core were kept at 4°C during migration studies to minimize leaching, (see Part I). Since preliminary experiments indicated the presence

of labelled viable organisms on the outer surface of the epoxy coating during the experiment, preventative measures were required. Thus, at the end of the migration experiment (48 hours), the core was removed from the core holder, swabbed with 1% Dettol in ethanol and flamed to kill any bacteria on the external surface. The core was then scored longitudinally at 0° and 180° of the circumference with a faceter diamond saw. The core was again swabbed with the disinfectant, flamed and split longitudinally using sterile strap iron and a hammer, (see Myers and McCready, 1964b).

The volume of the labelled cell suspension remaining in the lucite core holder at the end of the migration experiment was measured in order to calculate the amount which had passed into the core. A 10 ml volume of the remaining slurry was centrifuged and three 1 ml samples of the supernatant were removed and dried on planchets in order to determine the per cent of the radioactive label leached during the 48 hour migration period. Three 1 ml volumes of the remaining slurry were dried on planchets so that the amount of P^{32} remaining in the suspension could be calculated in order to estimate the number of cells which had migrated into the core.

Measurement of Bacterial Migration

a) Initially we attempted to follow the method described by Myers and McCready (1964b), to determine the rate of bacterial migration through the various cores. Readings were taken at each portal at 0 time (background reading) and after each 8 hour interval for 48 hours.

b) The freshly split cores were placed split surface down on thick 1.2% agar and pressed firmly into the agar. The core was immediately removed and the agar plate incubated for 24 hours.

The extent of migration was determined by measuring the distance from the end of the core which had been in contact with the labelled cell suspension to the upper limit of growth of Serratia marcescens.

c) The degree of penetration was also measured by determining the radioactivity at various locations on the split surface of the core. All radioactivity measurements were done with a G-M tube fitted with a plastic cap having a hole 1/8 inch in diameter through the centre. Counts on the split surface of cores were taken by placing the G-M tube in direct contact with the core surface at fixed locations along the longitudinal axis, (1/2", 1 1/4", 2" and 2 3/4" from the end of the core through which the slurry entered). At each location, radioactivity was measured for three minutes and recoreded as counts per minute in excess of background. Only counts 10% above background were considered significant.

d) Autoradiography, according to the method described by McCready (1963), was employed to show the presence of accumulated P^{32} in the core. The split surfaces of cores were allowed to remain in contact with x-ray film in a light tight box for a period of two weeks. The exposed x-ray film was removed in the dark and developed by a vividol developing solution. The negative was then used to make contact prints. (see Appendix A for developing procedures). Results of the migration studies are given in Tables XIII to XVII and illustrated in Plates 2 to 13.

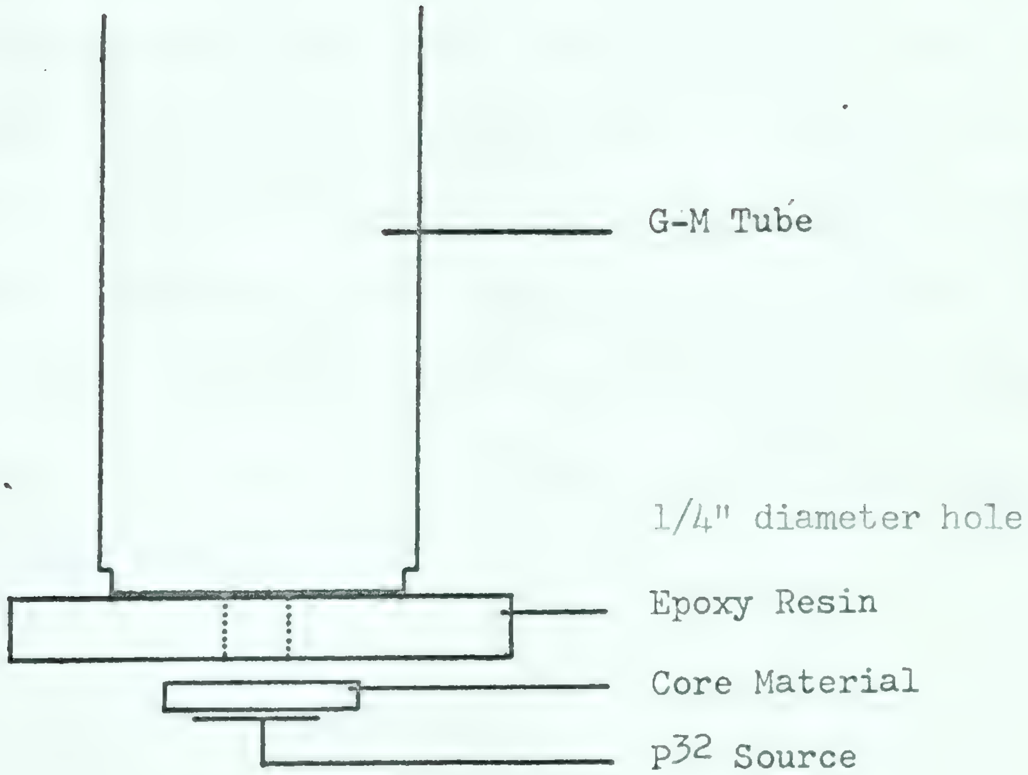
Estimation of Effect of Core Thickness on Radioactive Determinations

Absorption studies using various thicknesses of core material were done. A 1/4 inch thickness of epoxy resin through which a 1/4 inch diameter hole had been drilled was placed between the G-M tube and a source of P^{32}, (see Figure 4 for an illustration of the apparatus).

FIGURE 4

APPARATUS FOR MEASURING ABSORPTION OF

BETA RADIATION BY CORE MATERIAL



Different thicknesses of core material were placed between the radiation source and the epoxy resin and the disintegrations reaching the G-M tube were measured. The radiation reaching the G-M tube without any core material between the source and resin was recorded as the control. In the case of a P^{32} source, (a Beta-emitter), it is difficult to find the absolute thickness of core material which absorbs all the Beta particles emitted because of "bremsstrahlung". Bremsstrahlung is electromagnetic radiation which results during absorption of Beta particles when the Beta particles change their velocity in the field of the atomic nuclei. Due to the bremsstrahlung, a tailing of the curve is observed when one plots counts/minute against the core material thicknesses used for absorption. The end point or core material thickness for complete absorption is estimated by extrapolation from the linear portion of the curve. The results are shown in Table XI and the extrapolation in Figure 5.

TABLE XI

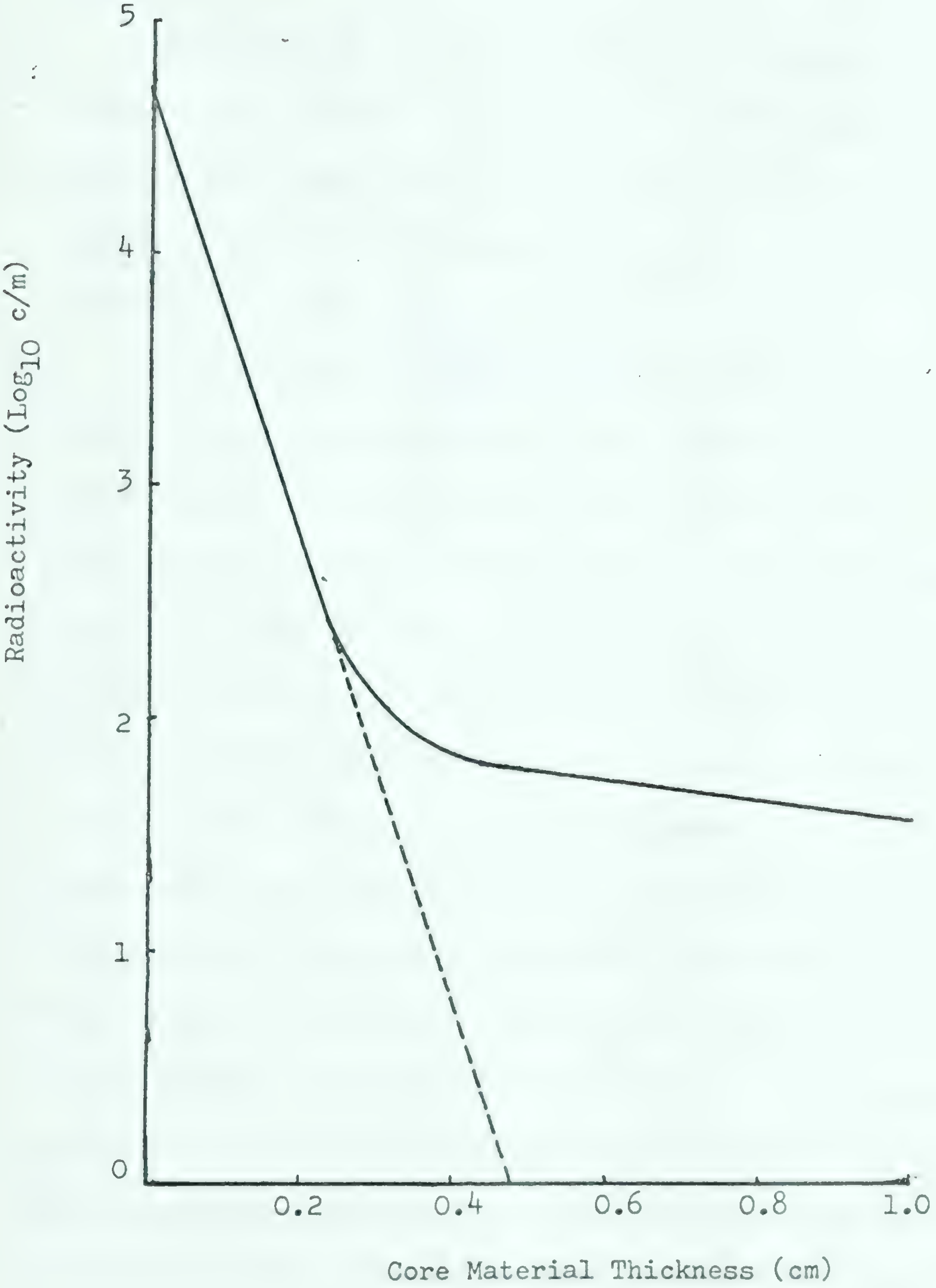
ESTIMATION OF THE EFFECT OF CORE MATERIAL THICKNESS ON RADIOACTIVE DETERMINATIONS

Thickness of core material (cm)	c/m
0	51,036
.15	1,778
.2	681
.25	174
.4	66
.5	59
1.0	37

FIGURE 5

ESTIMATION OF CORE MATERIAL THICKNESS

(CARDIUM SANDSTONE) WHICH COMPLETELY ABSORBS BETA PARTICLES



Effect of the Leached P^{32} on Autoradiograms

In order to place any emphasis on the autoradiograms as proof of bacterial migration, it is necessary to determine the effect of P^{32} which may have leached from the cells during the 48 hour period of migration.

Previously, we established that the P^{32} assimilation by the growing cells is approximately 40% of the P^{32} originally present in the medium, (see Table III). It was also established that a maximun of 8% of the P^{32} assimilated by the cells is lost during 48 hours, (see Table VII).

The effective porosity of a core will directly influence the volume of slurry which can enter the core and thus influence the radioactivity of the core whether due to assimilated or leached P^{32}. Based on the effective porosity of a given core, the volume of slurry required to fill the available pore space in a cube of the core material having a face area of 1 square cm. and a thickness of 0.48 cm. (complete absorption of radioactivity occurs with this thickness, see Figure 5), was calculated, (see Appendix D). The amount of P^{32} which could leach from the labelled cells into the supernatant of this volume of slurry was calculated. The results are shown in Table XII. These amounts of P^{32} were dried on 1 cm. square pieces of blotting paper. Radioactive counts emitted by the impregnated papers were recorded and the papers were then placed on a sandstone backing and put in direct contact with x-ray film in a light tight box for two weeks at which time the exposed film was developed. The autoradiogram obtained is seen in Plate 1. Figure 6 shows the amount of P^{32} dried on the papers and the locations of each impregnated

paper during exposure of the autoradiogram in Plate 1.

TABLE XII

AMOUNT OF P^{32} (μc)* WHICH COULD ENTER CORE VOLUMES (1 X 1 X 0.48 c.c.) OF VARIOUS EFFECTIVE POROSITIES

Per cent effective Pore Space	c.c. pore space per 0.48 c.c. core cube	μc P^{32}/pore space as leach	net c/m on dried surface
0	0	0	0
1	.0048	.00000864	4
2	.0096	.00001728	11
5	.0240	.0000432	21
10	.0480	.0000864	36
15	.0720	.0001296	60
20	.0960	.0001728	95

* Based on the range of per cent effective porosities, 40% of the available P^{32} being assimilated by the cells and 8% of the assimilated label being leached.

PLATE 1

EFFECT OF LEACHED P^{32} ON AUTORADIOGRAMS

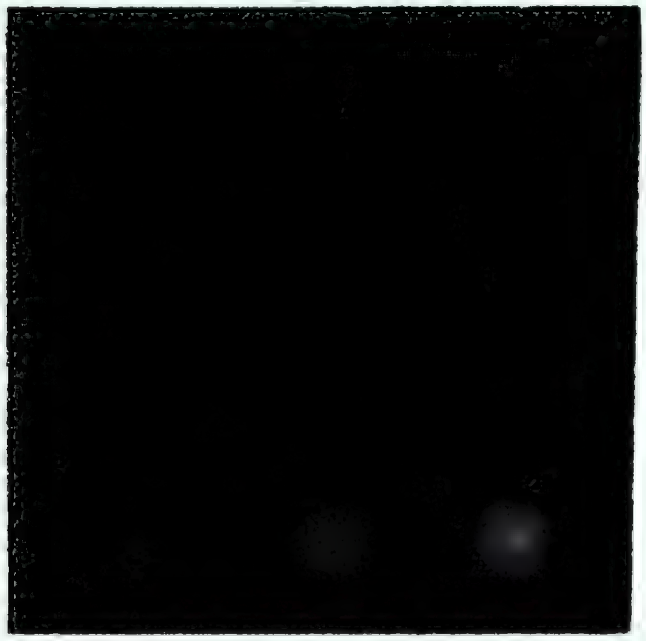
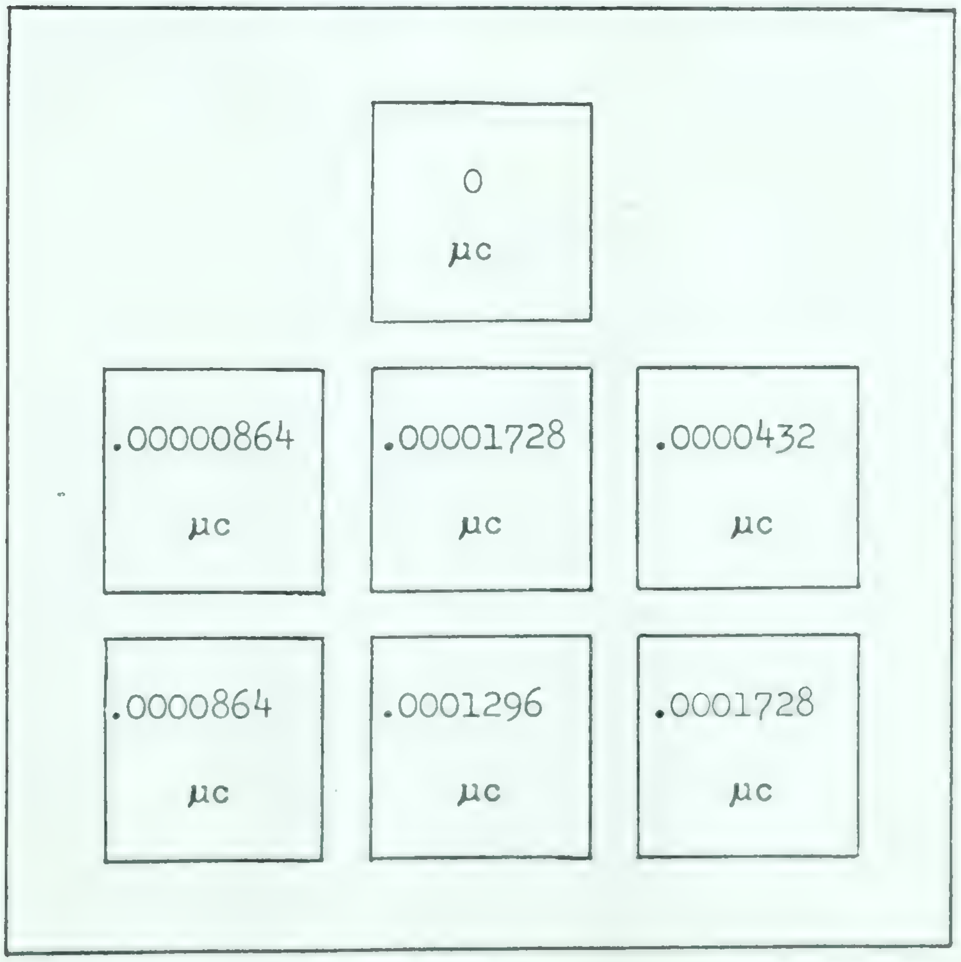


FIGURE 6

POSITION OF THE DIFFERENT AMOUNTS OF P^{32}

USED IN THE AUTORADIOGRAM SHOWN IN PLATE 1



RESULTS

Measurement of Bacterial Migration

In the initial experiments, it was found that labelled bacteria did not accumulate near the outer surface of the core to the extent that their presence could be detected by radioactive measurements taken at the portals drilled through the epoxy coating. The effect of the core material thickness on the radioactive determinations has also been noted, (see Table XII). Therefore, in subsequent experiments radioactivity measurements were made on the split surfaces of the cores.

Results obtained in the migration studies by: (1) replica growth of viable cells, (2) autoradiograms, and (3) radioactive measurements at the split surfaces are summarized in Tables XIII, XIV, XV, XVI and XVII. Autoradiograms indicating the locations of accumulated P^{32} at the split surfaces of the various cores are shown in Plates 2 to 13.

TABLE XIII

MIGRATION OF LABELLED *SERRATIA MARCESCENS* THROUGH TWINNING FIELD, PEKISKO FORMATION CORES

Core No.	Permeability md.	Oil Extraction	Number of cells migrated[1] (X 10^{-7})	Replica Growth (inches from bottom)	Plate Number of Autoradiogram[2]	Significant radioactivity on split surface[3] (inches from bottom)
1	less than 0.1	+	15.03	0.5	2	2.75
2	less than 0.1	–	5.69	top	0	N.A.
3	0.5	+	8.70	1.5	3	1.25
4	0.6	–	7.63	top	0	N.A.
5	34.0	+	31.95	2.25	0	0.5
6	41.0	–	4.80	1.0	0	N.A.
7	14.0	+	17.00	1.5	0	0
8	28.0	–	4.30	1.0	0	N.A.
9	13.0	–	2.08	0	0	N.A.
10	15.0	–	2.67	0	0	N.A.
11	9.5	+	2.21	0	0	0.5
12	9.9	–	1.57	0	0	N.A.
13	7.2	+	1.75	0	0	0.5
14	0.5	–	5.89	0	0	N.A.
15	6.6	–	4.51	0	0	N.A.

1. See Appendix E, Table 1 (e)
2. 0 = the developed autoradiogram gave no evidence for the accumulation of P^{32} labelled bacteria
3. See Appendix E, Table 1 (f)

\+ = Oil-extracted cores
– = Non-extracted cores
N.A. = Not available

PLATE 2

AUTORADIOGRAM OF SPLIT SURFACE OF

TWINNING FIELD, PEKISKO FORMATION CORE #1

PLATE 3

AUTORADIOGRAM OF SPLIT SURFACE OF

TWINNING FIELD, PEKISKO FORMATION CORE #3

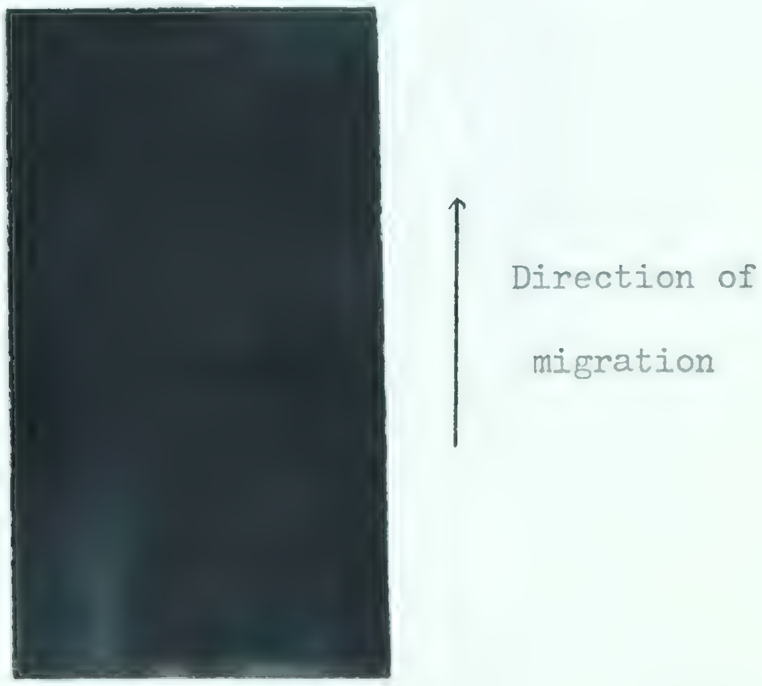


TABLE XIV

MIGRATION OF LABELLED *SERRATIA MARCESCENS* THROUGH PEMBINA FIELD, CARDIUM FORMATION CORES

Core No.	Permeability md.	Oil Extraction	Number of cells migrated[1] (X 10^{-9})	Replica Growth (inches from bottom)	Plate Number of Autoradiogram[2]	Significant radioactivity on split surface[3] (inches from bottom)
1	2.5	+	1.09	0.5	0	2.0
2	1.0	-	2.84	0	0	0.5
3	4.6	-	1.57	0	0	0
4	6.7	+	.19	0	0	0

1. See Appendix E, Table II (e)
2. 0 = the developed autoradiogram gave no evidence for the accumulation of P^{32} labelled bacteria.
3. See Appendix E, Table II (f)

+ = Oil-extracted cores

- = Non-extracted cores

TABLE XV

MIGRATION OF LABELLED *SERRATIA MARCESCENS* THROUGH SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORES

Core No.	Permeability md.	Oil Extraction	Number of cells migrated[1] (X 10^{-9})	Replica Growth (inches from bottom)	Plate Number of Autoradiogram[2]	Significant radioactivity on split surface[3] (inches from bottom)
1	1.7	-	36.46	top	4	0.5
2	less than 0.1	+	12.33	1.5	5	1.25
3	0.5	-	3.73	2.0	6	1.25
4	36.0	+	18.50	top	7	2.0
5	30.0	-	.32	0.5	0	1.25
6	306.0	-	11.53	1.5	0	2.0

1. See Appendix E, Table III (e)
2. 0 = the developed autoradiogram gave no evidence for the accumulation of P^{32} labelled bacteria
3. See Appendix E, Table III (f)

\+ = Oil-extracted cores
\- = Non-extracted cores

PLATE 4

AUTORADIOGRAM OF SPLIT SURFACE OF

SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORE #1

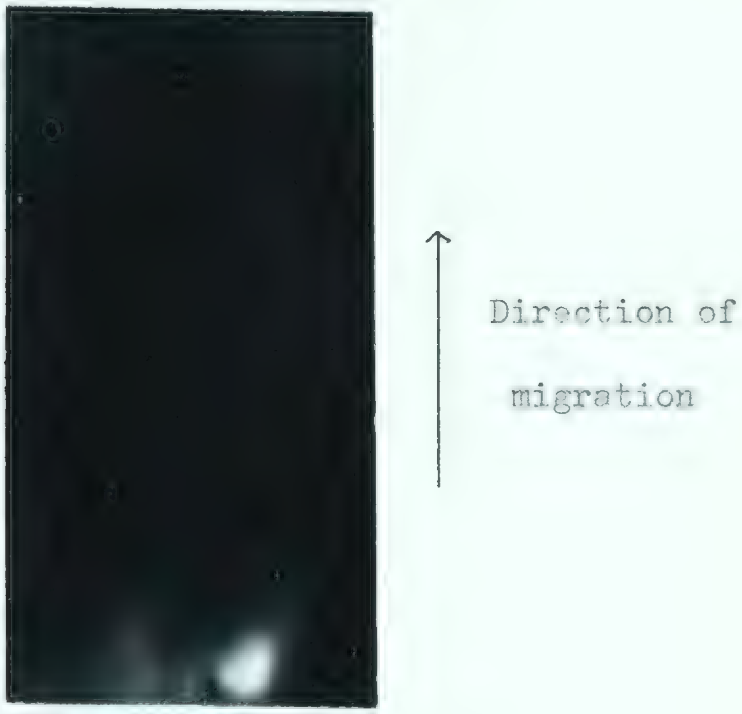


PLATE 5

AUTORADIOGRAM OF SPLIT SURFACE OF

SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORE #2

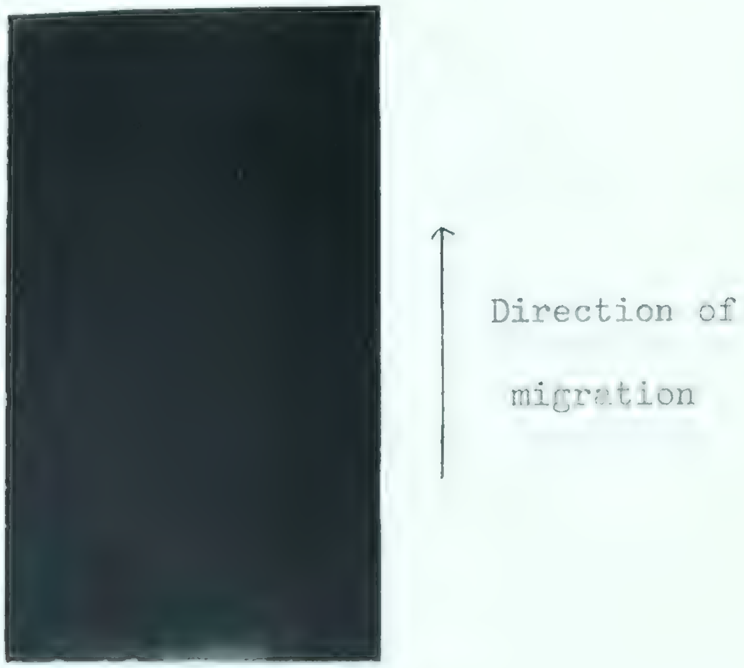


PLATE 6

AUTORADIOGRAM OF SPLIT SURFACE OF

SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORE #3

PLATE 7

AUTORADIOGRAM OF SPLIT SURFACE OF

SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORE #4

TABLE XVI

MIGRATION OF LABELLED SERRATIA MARSCESCENS THROUGH WILLESDEN GREEN FIELD, BELLY RIVER FORMATION CORES

Core No.	Permeability md.	Oil Extraction	Number of cells migrated[1] (X 10^{-9})	Replica Growth (inches from bottom)	Plate Number of Autoradio-gram[2]	Significant radioactivity on split surface[3] (inches from bottom)
1	6.6	-	15.05	0.75	0	N.A.
2	9.7	-	3.45	0.5	0	N.A.
3	less than 0.1	-	34.44	top	8	2.75
4	28.0	-	54.52	top	9	2.75
5	0.8	+	5.96	0.25	0	0.5

1. See Appendix E, Table IV (e)
2. 0 = the developed autoradiogram gave no evidence for the accumulation of P^{32} labelled bacteria
3. See Appendix E, Table IV (f)

+ = Oil-extracted cores
- = Non-extracted cores
N.A. = Not available

PLATE 8

AUTORADIOGRAM OF SPLIT SURFACE OF

WILLESDEN GREEN FIELD, BELLY RIVER FORMATION CORE #3

PLATE 9

AUTORADIOGRAM OF SPLIT SURFACE OF

WILLESDEN GREEN FIELD, BELLY RIVER FORMATION CORE #4

TABLE XVII

MIGRATION OF LABELLED SERRATIA MARCESCENS THROUGH BEAVERHILL LAKE FIELD, VIKING FORMATION CORES

Core No.	Permeability md.	Oil Extraction	Number of cells migrated[1] (X 10^{-9})	Replica Growth (inches from bottom)	Plate Number of Autoradiogram[2]	Significant radioactivity on split surface[3] (inches from bottom)
1	283.0	–	20.45	2.5	10	N.A.
2	168.0	+	26.04	1.25	11	1.25
3	143.0	–	32.90	less than 0.5	0	N.A.
4	118.0	–	18.22	2.0	12	2.0
5	3.2	–	20.08	1.5	13	0.5

1. See Appendix E, Table V (e)
2. 0 = the developed autoradiogram gave no evidence for the accumulation of P^{32} labelled bacteria
3. See Appendix E, Table V (f)

\+ = Oil-extracted cores

– = Non-extracted cores

N.A. = Not available

PLATE 10

AUTORADIOGRAM OF SPLIT SURFACE OF

BEAVERHILL LAKE FIELD, VIKING FORMATION CORE #1

PLATE 11

AUTORADIOGRAM OF SPLIT SURFACE OF

BEAVERHILL LAKE FIELD, VIKING FORMATION CORE #2

PLATE 12

AUTORADIOGRAM OF SPLIT SURFACE OF

BEAVERHILL LAKE FIELD, VIKING FORMATION CORE #4

PLATE 13

AUTORADIOGRAM OF SPLIT SURFACE OF

BEAVERHILL LAKE FIELD, VIKING FORMATION CORE #5

DISCUSSION

In order to carry out a definitive comparative study of the rate of bacterial migration through petroliferous formations, a great deal more information concerning the core material is required than was available (see Table IX). Only the ranges of effective pore space and the respective permeabilities were available. Since permeability is a measure of the rate of fluid flow through a substance and is not directly related to the effective pore space, these data are not sufficient. Thus, one might consider using per cent effective porosities as a parameter for a comparative study. However, there are certain characteristics of the types of pores which must also be considered and in some cases, these can not be measured unless the formation is split and studied by microscopy. The pore space of a rock can be divided into two kinds, the total pore space and the effective pore space. The total pore space is the total interstitial space and includes not only the communicating pores but any isolated pores that may exist, whereas the effective pore space includes only the communicating pores. The effective pore space is relative, depending on such factors as the constitutions of the liquid used for measurement, the size of the pores, the material of the rock, temperature and pressure. In a study such as this, one would be concerned with the effective pore space available to water under atmospheric pressure at 4°C.

Considering either permeability or porosity as a parameter for comparison of migration rates, it is also desirable to know something of the types of pore spaces involved. These may be classified as (a) interparticulate, (b) vuggy and (c) fractures. The interparticulate

(either intergranular or intercrystalline) porosity is usually made up of tortuous tubules of varying diameters sometimes completely pinched off at one or both ends. The vugs are cavities that are distinctly larger than the size of the particles comprising the rock matrix and may either be associated with the tortuous tubules or be isolated. The presence of fractures has a great effect on the permeability and the effective porosity of a core. For example, a fracture could increase the permeability of a porous and otherwise relatively impermeable core many fold. The fractures found in any formation may or may not be present in the cores choosen for study.

Not only must the physical characteristics of a formation be considered but also the chemical content as well, especially the amount and type of clay that may be present. In the study described here, dried cores were used. If clay which swells when esposed to distilled water was present in the core spaces, it would attract water at the beginning of the migration study and swell, thus plugging the core so that the bacterial migration would completely stop or at least be greatly decreased, (see Fekete, 1959). Clay content is mainly associated with sandstone formations. Samples from each formation were analyzed for expandable clay content.* Belly River, Viking and Cardium formations contained expandable clay materials which could affect bacterial migration. Pekisko formation had no swelling clays while Beaverhill Lake formation contained only traces. The complete analyses for expandable clay content in the different formations are given in detail in Appendix F.

* The analyses were done by Bradford Laboratories, A Division of Calgon Corporation (Canada) Limited, Edmonton, Alberta.

The "silica effect" may also cause some plugging in the sandstone cores, (see Merkt, 1943).

The per cent effective porosity of most reservoir formations ranges from 5 to 40 per cent and is most commonly between 10 and 20 per cent. Carbonate reservoirs generally have a slightly lower porosity than sandstone reservoirs but the permeability of carbonate rocks may be higher, (see Levorsen, 1954). In sandstone reservoirs, the plugging effect caused by the swelling of clay may be the cause of reduced permeability. Samples from cores with the lowest permeability and those with the highest permeability from each formation used in our studies were analyzed for per cent effective pore space, (see Table IX).

For a complete study of the characteristics mentioned, it would be necessary to analyze each core from the different formations since both the physical characteristics and the clay content would vary according to the depth from which the cores are taken, (see Waring and Layer, 1954).

Since many of the above mentioned characteristics are not known for our cores, our experimental results only indicate whether or not bacteria can migrate through the petroliferous formations and the effect of the presence of oil on such migration.

Carbon tetrachloride was used as the sole solvent for the extraction of the core oil. Using the method described, it can be said that all the core oil available to and extractable by carbon tetrachloride was removed. It is apparent from a study of the results shown in Table X that the extraction procedure did not leave any residues, in any of the cores, which were toxic to the bacteria

during 48 hours exposure. The non-extracted core materials also did not show any toxicity toward the bacteria in the same length of time.

From the results in Table XI and Figure 5, it is evident that a large percentage of the Beta particles are easily stopped by even a 0.15 cm. thickness of the core material. Complete absorption is estimated to occur when the core material thickness is 0.48 cm. Beta particles are almost entirely slowed down by their interaction with atomic electrons in the matter through which they pass. The number of electrons per unit volume is very nearly proportional to the mass for the lighter elements in the Periodic Table (see Kamen, 1957). For this study, Cardium Sandstone was used since it has an intermediate density as compared to the other formations.

From the results shown in Plate I and the estimations in Table XII, it is suggested that if the assimilation by the cells is 40% (3 μc) of the available P^{32} and the cells lose 8% (0.24 μc) of the assimilated P^{32} into the slurry supernatant, the leached P^{32} could have an effect on the autoradiogram of the split surface of a test core which has an effective porosity of greater than 10%. From further examination of Plate 1 and the range of effective porosities for the different formations (see Table IX), it is evident that the leached P^{32} will have a limited effect in giving false postive results with autoradiograms of the split surface of some of the cores studied. However, it must be pointed out that the results shown in Plate 1 depict the maximum effect of the leached P^{32} which could enter the effective pore space since the amount of P^{32} was dried on blotting paper and placed in direct contact with the x-ray film. Under these conditions, almost all of the P^{32} present would

be functional in exposing the x-ray film. Actually, under the experimental conditions when the autoradiograms are prepared, there would be a considerable amount of absorption of the Beta particles emitted from the P^{32} furthest removed from the split surface of the core, (see Table XI and Figure 5).

Our results show that there is a high absorption of Beta particles by the core materials used, (see Table XI and Figure 5), and that only small volumes of the radioactive slurrys entered the cores, (see Appendix E). These findings could explain the fact that radiation could not be detected at the portals in the epoxy coating. Myers and McCready, (1964b), were able to use this method of tracing bacterial migration effectively in cores of Berea Sandstone which have relatively large effective porosities and high permeabilities as compared to the cores used in the present experiments.

From the studies on the leaching of P^{32} by labelled cells (see Table VIII), it is evident that cells which are grown in medium with a higher specific activity assimilate more P^{32} and subsequently leach more. Thus, when using labelled cells for studying bacterial migration, it is necessary to arrive at a balance where the maximum amount of P^{32} is used in the medium so that the labelled cells will have a detectable radioactivity and yet have a specific activity low enough that the P^{32} leached from the cells will have only a minimal effect in giving false positive results in autoradiograms. Using autoradiograms for determining the extent of bacterial penetration has an advantage over the replica growth method in that the presence of accumulated labelled bacteria just beneath the split surface of the cores can be detected when the replica growth plate prepared from the suraface gives a completely negative result, (See Table XIII, Cores #11

and #13). The method of determining the extent of bacterial penetration by taking radioactivity counts along the split surface of the core is a little more accurate than the autoradiograms since it detects a lower concentration of radioactivity. However, both methods require the labelled bacteria to accumulate to a relatively high concentration at any given location, whereas the replica growth method will detect very small numbers of viable bacteria on the split surface. It must be emphasized that with the latter method, the bacteria must be viable and present at the surface to give a positive result.

From the results summarized in Tables XIII to XVII and given in detail in Appendix E, it is apparent that the test organism, Serratia marcescens, can penetrate and migrate through (within 48 hours at atmospheric pressure) cores taken from various petroliferous formations found in Alberta (see Table XIII, Core Nos. 2 and 4; Table XV, Core Nos. 1 and 4; Table XVI, Core Nos. 3 and 4). There is no apparent correlation between permeability, effective porosity or the presence of oil and the extent of bacterial migration during 48 hours in the cores studied.

Different species of viable bacteria have been shown to be present in various petroliferous formations at different depths, (Zobell, 1952, 1959). It has been suggested that these bacteria were either indigenous to the oil bearing formation or had been introduced during drilling operations and became acclimatized to the higher pressures and temperatures. The possibility of bacteria being carried into the formation by the movement of ground water has also been suggested. Our experimental results show that bacteria which have

been found in cores taken from oil bearing formations could have migrated from another location provided required nutrient was present and suitable conditions of temperature and pressure existed.

The presence of bacteria in petroliferous formations can be advantageous to the petroleum industry. Robinson (1963), discusses evidence for the biogenesis of petroleum hydrocarbons which is most clear in the younger oils. Since oil originates from organic matter and most organic matter is susceptible to microbial modifications, it is almost axiomatic that bacteria contribute to the process. Zobell (1950), gives evidence for the bacterial production of certain hydrocarbons and Neave and Buswell (1928), recorded the bacterial production of ethane in small quantities. Davis and Squires (1954), state that among gases produced by microbial fermentation of cellulose are large quantities of methane, from 3.2 to 7 p.p.m. ethane, 3.0 to 4.7 p.p.m. ethylene, 0.66 to 0.14 p.p.m. propane and 0.13 to 0.21 p.p.m. propylene. Evidence that from 0.03 to 0.95 per cent of the carbon content of certain bacterial cells from marine materials consists of liquid and solid hydrocarbons was reported by Stone and Zobell (1952). Smith (1954), claims that this amount is adequate to account for the hydrocarbon content of recent marine sediments.

An increase in the amount of liquid and solid hydrocarbons recoverable from diatoms undergoing degradation by anaerobic bacteria has been recorded by Clarke and Mazur (1941). Part of this hydrocarbon may have been produced by the bacteria but most of it was probably released from the diatom matrix by bacterial decomposition. Similarly, bacteria are believed to be instrumental in the release of bound hydrocarbons from the decomposing remains of plants and animals,

many of which are known to contain hydrocarbons, (see Chibnall et al., 1934).

Zobell (1947 a, b), suggests several ways in which bacteria aid in the liberation of oil from oil bearing materials and promote its flow or accumulation in reservoirs. These are : (1) Dissolution of the organic matrix by the bacteria or acids produced by them resulting in improved permeability of the rock favoring the movement or accumulation of oil, (2) Carbon dioxide, methane and hydrogen produced by bacteria promote the flow of oil by reduction of the viscosity of the oil by solution in the oil and by increasing the gas pressure, (3) Production of detergents by the bacteria (perhaps by the oxidation of hydrocarbons in the oil) which help to remove the oil, (4) Growing bacteria can tenaciously attach themselves to a solid surface and replace any oil that may have been there, and (5) Bacteria may also reduce the viscosity of the oil by direct chemical action upon the oil.

On the other hand, the presence of certain micro-organisms in a petroliferous formation can be very costly to the petroleum industry. The oxidation of hydrocarbons by numerous species of bacteria, yeasts and molds is discussed in reviews by Zobell (1946, 1959) and Foster (1962). Other species of bacteria which normally lack the ability to attack hydrocarbons have been shown to adapt themselves to do so when exposed to oils for different lengths of time.

Ashirov and Sazonova (1962), discuss the sealing of oil deposits in carbonate reservoirs as a result of the action of sulfate reducing bacteria. Their experiments confirmed that the rock microflora, particularly the group of sulfate-reducing bacteria at the oil-water

contact can, by their vital activity, produce secondary calcite, which filling pores and cracks, forms an impermeable layer isolating the pool from the underlying rock waters and adversely affecting the permeability of the reservoir.

It has been found that the so-called iron bacteria play a role in the corrosion of pipes and other iron structures in soil and water (see Doig and Wachter, 1951). The accomplish this by first utilizing ferrous bicarbonate, originating from the action of aqueous carbon dioxide on commerical iron and then producing a crust which separates regions where reducing processes are prevalent from those where oxidation takes place, (see von Wolzogen Kuhr, 1937, Starkey, 1945; Pringsheim, 1949, 1952; Updegraff, 1955; and Sharpley, 1961).

CONCLUSIONS

(1) The results presented here conclusively show that bacteria can migrate through petroliferous formations whether oil is present or not.

(2) Although the extent of migration must depend on porosity and permeability, our results do not indicate any correlation between the degree of porosity or permeability and the extent to which the bacteria will migrate during a 48 hour period. Indeed, it seems to be almost impossible to make a true comparative study of the rates of bacterial migration through petroliferous formations due to a lack of knowledge of many of the characteristics of the formation which could obviously influence migration.

(3) Because the different methods used for studying the bacterial migration measure different things or the same thing in different ways, complete correlation of the results from the different methods can not be expected, (e.g. replica growth and autoradiograms). However, each method is essential to the complete study in that it provides some information which is not otherwise evident.

RECOMMENDATION

During the month of March, 1964, 13,963,805 barrels of water* were injected into the different petroliferous formations of Alberta to aid oil recovery. Since water injection has continually been increasing in popularity as a method for oil recovery, there have been numerous papers published on the plugging of formations by bacteria present in the injection water, Lada, 1959; Beck, 1946, 1947; and Merkt, 1943.

Numerous injection waters used in Alberta oil fields have been studied by Myers and Slabyj (1962), and found to be highly contaminated with various micro-organisms which can be detrimental to the petroliferous formation (see Lada, 1959). Since the present study shows that bacteria introduced into a formation are capable of migrating some distance through the formation and accumulating at various places where they could produce undesirable effects, it is suggested that greater attention be given to microbiological control of injection waters.

* See "Monthly Statistics", Alberta Oil and Gas Industry, March, 1964. Oil and Gas Conservation Board, Calgary, Alberta.

APPENDIX A

MATERIALS

Bacto Nutrient Broth

Peptone	5 Gm
Beef Extract	3 Gm
Distilled Water add to	1000 ml

Glucose .... Fisher Scientific Company
    Reagent Grade, anhydrous d-glucose cat. No. D. 16

Celite 281 .... John Manville Company
    Amorphous Diatomaceous Silica Powder.

Epon Resin 828 .... Shell Chemical Company, a
    division of Shell Oil Company, Houston, Texas.

Epon Curing Agent T-1..... Shell Chemical Company, a
    division of Shell Oil Company, Houston, Texas.

Kodak Medical X-Ray Film.

F-3, Single weight paper for contact prints.

PHOTOGRAPHY

1. Developing Autoradiograms

The exposed x-ray film was intermittently agitated in a 1:1 Ansco Vividol developer with water solution for five minutes, neutralized in 1% acetic acid short stop bath for five seconds and finally fixed in the Ansco Acid Fixer with hardener for five minutes.

2. Developing Contact Prints

The exposed film paper was intermittently agitated in a 1:2 Ansco Vividol developer with water solution for 2½ minutes, neutralized in a 1% acetic acid short stop bath for five seconds and fixed in the Ansco Acid Fixer with hardener for five minutes.

APPENDIX B

I EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS GROWN IN ROUX BOTTLES AT 30°C

TABLE I (a)

MEASUREMENT OF THE RADIOACTIVITY IN THE GROWTH MEDIA

Sample	µc P^{32}/150 ml media	c/m* ml media 1	c/m* ml media 2	c/m* ml media 3	Average c/m* ml media	Calulated c/m* 150 ml media
1	0	0	0	0	0	0
2	5.0	2994	3073	2763	2944	441,600
3	7.5	4223	4783	4461	4489	673,350
4	10.0	6203	5457	6007	5889	883,350
5	12.5	7986	7783	7943	7904	1,185,600

* radioactivity in excess of background

TABLE I (b)

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 8 HOURS INCUBATION

Sample	c/m* ml cell suspension 1	c/m* ml cell suspension 2	c/m* ml cell suspension 3	Average c/m* ml cell suspension	c/m* 150 ml cell suspension	Per cent P^{32} assimi-lation	Viable cell count/ml X 10^{-9}	Radio-activity c/m* 10^{9} cells
1	0	0	0	0	0	0	4.80	0
2	690	650	712	684	102,600	23.23	4.85	141.03
3	976	1012	1000	996	149,400	22.19	4.83	206.21
4	1271	1386	1345	1334	200,100	22.65	4.74	281.58
5	1752	1718	1582	1684	252,600	21.31	4.77	353.14

* radioactivity in excess of background

II EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS GROWN IN FLASKS IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C.

TABLE II (a)

MEASUREMENT OF THE RADIOACTIVITY IN THE GROWTH MEDIA

Sample	μc P^{32}/150 ml media	c/m* ml media			Average c/m* ml media	Calculated c/m* 150 ml media
		1	2	3		
1	0	0	0	0	0	0
2	5.0	3916	3922	3933	3924	588,600
3	7.5	6093	6014	6230	6112	916,800
4	10.0	7708	7640	7663	7670	1,150,500
5	12.5	10002	10123	10335	10153	1,522,950

* radioactivity in excess of background

TABLE II (b)

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED AND THE VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 8 HOURS INCUBATION

Sample	c/m* ml cell suspension			Average c/m* ml cell suspension	c/m* 150 ml cell suspension	Per cent P^{32} assimilation	Viable cell count/ml X 10^{-9}	Radioactivity c/m* 10^9 cells
	1	2	3					
1	0	0	0	0	0	0	8.6	0
2	1570	1536	1528	1545	243,947	41.44	8.75	185.88
3	2395	2352	2389	2379	375,632	40.97	8.87	242.32
4	3152	3057	3019	3076	485,684	42.21	8.5	280.92
5	3729	3560	3857	3715	586,579	38.51	8.4	465.46

* radioactivity in excess of background

1 - time corrected values (time factor = 0.95)

III EFFECT OF THE CONCENTRATION OF GLUCOSE IN THE GROWTH MEDIUM ON THE VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY *SERRATIA MARCESCENS*

TABLE III (a)

MEASUREMENT OF RADIOACTIVITY IN THE GROWTH MEDIA (5 μc P^{32}/150 ml)

Sample	Per cent w/v Glucose	c/m* ml media			Average c/m* ml media	c/m* 150 ml media
		1	2	3		
1	0	2307	2343	2429	2360	354,000
2	0.1	2299	2421	2321	2347	352,050
3	0.2	2412	2305	2362	2360	354,000
4	0.3	2235	2317	2192	2248	337,200
5	0.4	2274	2297	2366	2312	346,800
6	0.5	2194	2218	2221	2211	331,650
7	0.6	2063	2262	2132	2152	322,800
8	0.7	2210	2026	2132	2123	318,450
9	0.8	1999	2138	2054	2064	309,600
10	0.9	2014	2085	2073	2057	308,550
11	1.0	2116	2118	1961	2065	309,750

* radioactivity in excess of background

TABLE III (b)

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED AND VIABLE CELL COUNT OF *SERRATIA MARCESCENS* AFTER 8 HOURS GROWTH IN NUTRIENT BROTH CONTAINING DIFFERENT CONCENTRATIONS OF GLUCOSE

Sample	c/m* 5 ml cell suspension 1	c/m* 5 ml cell suspension 2	c/m* 5 ml cell suspension 3	Average c/m* 5 ml cell suspension	Average c/m* 150 ml cell suspension	Per cent P^{32} assimilation	Viable cell count/ml X 10^{-9}	Radioactivity c/m* 10^9 cells
1	3136	2583	3130	2950	88,500	25.00	4.74	124.47
2	3555	3483	3491	3510	105,300	29.91	4.93	142.39
3	3837	3763	3729	3776	113,280	32.11	4.87	155.07
4	4058	4285	4036	4126	123,780	36.71	5.09	162.12
5	4514	4226	4568	4436	133,080	38.37	5.43	163.38
6	4745	4436	4597	4593	137,790	41.55	5.51	166.71
7	4596	4540	4432	4523	135,690	42.04	5.67	159.55
8	4035	4090	4077	4067	122,010	38.31	5.82	139.76
9	4859	4137	3904	3967	119,010	38.44	5.79	137.03
10	3972	3310	3585	3622	108,660	35.22	5.76	125.76
11	3474	3290	3321	3362	100,860	32.56	5.79	116.13

* radioactivity in excess of background

IV EFFECT OF TIME ON THE VIABLE CELL COUNT AND THE AMOUNT OF P^{32} ASSIMILATED BY SERRATIA MARCESCENS INCUBATED IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C

TABLE IV a

MEASUREMENT OF RADIOACTIVITY IN THE GROWTH MEDIUM

Sample test medium	c/m* ml medium 1	2	3	average c/m* ml medium	calculated c/m* 210 ml medium
7 µc P^{32}/210 ml	2604	2667	2613	2628	551,880

* radioactivity in excess of background

TABLE IV b

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED AND THE VIABLE CELL COUNT OF *SERRATIA MARCESCENS* AFTER VARIOUS INTERVALS OF INCUBATION AT 30°C

Hours of Incubation	c/m* ml cell suspension 1	2	3	Average c/m* ml cell suspension	c/m* total cell suspension	Per cent P^{32} assimilation	Viable cell count/ml control	test	Radioactivity c/m* 10 cells
0	0	0	0	0	0	0	9.2 X 10^{6}	9.5 X 10^{6}	0
1	0	0	0	0	0	0	1.09 X 10^{7}	1.17 X 10^{7}	0
2	0	0	0	0	0	0	3.72 X 10^{7}	3.63 X 10^{7}	0
3	0	2	11	4	840	0.15	8.73 X 10^{7}	9.76 X 10^{7}	40.98
4	12	8	11	10	2,100	0.38	2.27 X 10^{8}	2.13 X 10^{8}	46.95
5	37	43	48	43	9,030	1.64	7.43 X 10^{8}	7.73 X 10^{8}	55.63
6	179	198	191	189	39,690	7.19	2.52 X 10^{9}	2.96 X 10^{9}	63.85
7	501	497	513	504	105,840	19.18	5.02 X 10^{9}	5.19 X 10^{9}	97.11
8	1036	992	1029	1019	213,990	38.77	5.83 X 10^{9}	5.99 X 10^{9}	170.12
9	1033	1097	1075	1068	224,280	40.64	6.32 X 10^{9}	6.27 X 10^{9}	170.33
10	1105	1048	1035	1063	223,230	40.45	6.17 X 10^{9}	6.33 X 10^{9}	167.93
11	1088	972	1068	1043	219,030	39.69	6.29 X 10^{9}	6.23 X 10^{9}	167.42
12	1078	1096	996	1057	221,970	40.22	6.26 X 10^{9}	6.31 X 10^{9}	167.51
13	1001	1086	1008	1032	216,720	39.27	6.34 X 10^{9}	6.29 X 10^{9}	164.07
14	1038	1022	1062	1041	218,610	39.61	6.26 X 10^{9}	6.27 X 10^{9}	166.03

* radioactivity in excess of background

V PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED SERRATIA MARCESCENS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUIDS WERE THE WATER SUPERNATANTS FROM SUSPENSIONS OF THE VARIOUS OIL-BEARING FORMATIONS).

TABLE V a

MEASUREMENT OF RADIOACTIVITY IN THE GROWTH MEDIA CONTAINING 7.5 µc P^{32}/150 ML.

Flask number	c/m* ml medium			Average c/m* ml medium	c/m* 150 ml medium
	1	2	3		
1	3246	3333	3258	3249	491,850
2	3223	3187	3253	3221	483,150
3	3377	3171	3161	3236	485,400
4	3377	3156	3183	3239	485,850
5	3359	3117	3209	3228	484,200

* radioactivity in excess of background.

TABLE V b

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED BY *SERRATIA MARCESCENS*

AFTER 8 HOURS INCUBATION IN FLASKS IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C.

Flask number	Source of supernatant for cell suspension	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Per cent P^{32} assimilation
		1	2	3			
1	Belly River	2086	1983	1863	1977	197,700	40.19
2	Viking	1987	1835	1864	1895	189,500	39.22
3	Cardium	1979	1907	2282	2056	205,600	42.36
4	Pekisko	1986	1939	2068	1998	199,800	41.12
5	Beaverhill Lake	2033	2011	1841	1962	196,200	40.52

* radioactivity in excess of background

TABLE V c

PER CENT RADIOACTIVITY LOST FROM LABELLED SERRATIA MARCESCENS CELLS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND WILLESDEN GREEN FIELD, BELLY RIVER FORMATION)

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	4	7	6	6	600	667	0.34
12	.90	56	65	52	58	5,800	6,444	3.36
24	.90	102	110	92	101	10,100	11,222	5.68
36	.90	131	126	138	132	13,200	14,667	7.42
48	.82	150	155	141	149	14,900	18,171	9.19
60	.82	192	190	207	196	19,600	23,902	12.09
72	.82	286	263	270	273	27,300	33,292	16.84
84	.82	290	326	319	312	31,200	38,049	19.25
96	.82	360	351	340	350	35,000	42,805	21.65

* radioactivity in excess of background

TABLE V d

PER CENT RADIOACTIVITY LOST FROM LABELLED *SERRATIA MARCESCENS* CELLS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND BEAVERHILL LAKE FIELD, VIKING FORMATION.)

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	4	8	8	7	700	777	0.41
12	.90	50	56	50	52	5,200	5,777	3.04
24	.90	92	98	90	93	9,300	10,333	5.45
36	.90	125	122	131	126	12,600	14,000	7.39
48	.82	140	152	130	141	14,100	17,195	9.07
60	.82	175	176	182	178	17,800	21,707	11.45
72	.82	232	250	261	248	24,800	30,122	15.89
84	.82	279	290	308	292	29,200	35,609	18.79
96	.82	336	351	348	345	34,500	42,073	22.20

* radioactivity in excess of background

TABLE V e

PER CENT RADIOACTIVITY LOST FROM LABELLED *SERRATIA MARCESCENS* CELLS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND PEMBINA FIELD, CARDIUM FORMATION.)

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	12	13	19	15	1,500	1,667	0.81
12	.90	94	82	99	92	9,200	10,222	4.97
24	.90	124	138	120	127	12,700	14,111	6.86
36	.90	158	138	134	143	14,300	15,889	7.73
48	.82	174	186	188	183	18,300	22,319	10.85
60	.82	220	212	219	217	21,700	26,463	12.87
72	.82	268	287	273	276	27,600	33,659	16.37
84	.82	320	332	290	314	31,400	38.293	18.62
96	.82	338	368	342	349	34,900	42,561	20.70

* radioactivity in excess of background

TABLE V f

PER CENT RADIOACTIVITY LOST FROM LABELLED *SERRATIA MARCESCENS* CELLS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND TWINNING FIELD, PEKISKO FORMATION.)

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	17	15	7	13	1,300	1,444	0.72
12	.90	73	65	74	71	7,100	7,889	3.95
24	.90	91	106	94	97	9,700	10,777	5.39
36	.90	143	159	138	147	14,700	16,333	8.17
48	.82	171	173	182	175	17,500	21,341	10.68
60	.82	187	211	223	207	20,700	25,244	12.63
72	.82	267	287	261	272	27,200	33,171	16.60
84	.82	323	346	322	330	33,000	40,244	20.14
96	.82	362	383	356	367	36,700	44,756	22.40

* radioactivity in excess of background

TABLE V g

PER CENT RADIOACTIVITY LOST FROM LABELLED SERRATIA MARCESCENS CELLS AT ROOM TEMPERATURE (23-25°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION.)

Hours of Suspension	Time factor	c/m* ml suspension 1	2	3	Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
0	.90	14	18	18	17	1,700	1,889	0.96
12	.90	77	80	79	79	7,900	8,778	4.47
24	.90	104	102	96	101	10,100	11,222	5.72
36	.90	138	130	132	133	13,300	14,778	7.53
48	.82	144	152	139	145	14,500	17,683	9.01
60	.82	182	190	206	193	19,300	23,536	11.99
72	.82	252	268	239	253	25,300	30,854	15.72
84	.82	306	332	294	311	31,100	37,927	19.33
96	.82	358	361	312	344	34,400	41,951	21.38

* radioactivity in excess of background

VI PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED *SERRATIA MARCESCENS* AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUIDS WERE THE WATER SUPERNATANTS FROM SUSPENSIONS OF THE VARIOUS OIL-BEARING FORMATIONS).

TABLE VI a

MEASUREMENT OF RADIOACTIVITY IN THE GROWTH MEDIA CONTAINING 7.5 μc P^{32}/150 ML.

Flask number	c/m* ml medium 1	2	3	Average c/m* ml medium	c/m* 150 ml medium
1	3185	3223	3341	3250	487,500
2	3201	3100	3359	3220	483,000
3	2970	2989	3029	2996	449,400
4	3150	3170	3176	3165	474,750
5	2930	2963	2962	2952	442,800

* radioactivity in excess of background.

TABLE VI b

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED BY <u>SERRATIA MARCESCENS</u>

AFTER 8 HOURS INCUBATION IN FLASKS IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C.

Flask number	Source of supernatant for cell suspension	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Per cent P^{32} assimilation
		1	2	3			
1	Belly River	1724	1820	1775	1773	177,300	36.37
2	Viking	2011	2012	1690	1904	190,400	39.42
3	Cardium	1810	1794	1839	1814	181,400	40.36
4	Pekisko	1908	1874	1913	1898	189,800	39.98
5	Beaverhill Lake	1804	1818	1786	1803	180,300	40.72

* radioactivity in excess of background

TABLE VI c

PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED *SERRATIA MARCESCENS* CELLS AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND WILLESDEN GREEN FIELD, BELLY RIVER FORMATION).

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	19	13	15	16	1,600	1,778	1.00
12	.90	67	63	85	72	7,200	8,000	4.51
24	.90	79	81	69	76	7,600	8,444	4.76
36	.90	94	107	104	102	10,200	11,333	6.39
48	.90	119	101	129	116	11,600	12,889	7.27
60	.90	128	142	149	140	14,000	15,556	8.77
72	.82	127	125	115	122	12,200	14,878	8.39
84	.82	149	131	145	142	14,200	17,317	9.77
96	.82	143	149	141	144	14,400	17,562	9.91

* radioactivity in excess of background.

TABLE VI d

PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED SERRATIA MARCESCENS CELLS AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND BEAVERHILL LAKE FIELD, VIKING FORMATION).

Hours of Suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.90	21	12	14	16	1,600	1,778	0.93
12	.90	87	89	83	86	8,600	9,556	5.02
24	.90	97	87	91	92	9,200	10,222	5.37
36	.90	123	131	129	128	12,800	14,222	7.47
48	.90	127	123	134	128	12,800	14,222	7.47
60	.90	142	155	141	146	14,600	16,222	8.52
72	.82	153	157	146	152	15,200	18,537	9.74
84	.82	177	161	179	172	17,200	20,976	11.02
96	.82	179	167	183	176	17,600	21,463	11.27

* radioactivity in excess of background.

TABLE VI e

PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED SERRATIA MARCESCENS CELLS AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND PEMBINA FIELD, CARDIUM FORMATION).

Hours of suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.95	15	17	14	15	1,500	1,579	0.87
12	.95	66	76	64	69	6,900	7,263	4.00
24	.95	78	82	70	83	8,300	8,737	4.82
36	.95	92	88	80	87	8,700	9,158	5.05
48	.95	116	108	116	113	11,300	11,894	6.56
60	.82	112	118	116	115	11,500	14,024	7.73
72	.82	132	130	136	133	13,300	16,219	8.94
84	.82	158	145	146	150	15,000	18,292	10.08
96	.82	162	166	150	159	15,900	19,390	10.69

* radioactivity in excess of background.

TABLE VI f

PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED SERRATIA MARCESCENS CELLS AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND TWINNING FIELD, PEKISKO FORMATIONS).

Hours of suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.95	14	16	17	16	1,600	1,684	0.89
12	.95	76	66	71	71	7,100	7,474	3.94
24	.95	86	84	72	81	8,100	8,526	4.49
36	.95	84	78	97	86	8,600	9,053	4.77
48	.95	102	120	121	114	11,400	12,000	6.32
60	.82	118	110	123	117	11,700	14,268	7.52
72	.82	136	135	123	131	13,100	15,976	8.42
84	.82	150	158	146	151	15,100	18,414	9.70
96	.82	166	165	148	160	16,000	19,512	10.28

* radioactivity in excess of background

TABLE VI g

PER CENT RADIOACTIVITY LOST FROM P^{32} LABELLED SERRATIA MARCESCENS CELLS AT REFRIGERATOR TEMPERATURE (4°C) AS A FUNCTION OF TIME. (SUSPENDING FLUID WAS THE WATER SUPERNATANT FROM THE SUSPENSION OF GROUND SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION).

Hours of suspension	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent radioactivity leached
		1	2	3				
0	.95	12	14	19	15	1,500	1,579	0.87
12	.95	66	62	75	68	6,800	7,158	3.97
24	.95	82	78	76	79	7,900	8,316	4.61
36	.95	88	90	81	86	8,600	9,053	5.02
48	.95	110	116	108	111	11,100	11,684	6.48
60	.82	100	128	113	114	11,400	13,902	7.71
72	.82	130	136	126	131	13,100	15,976	8.86
84	.82	152	140	156	149	14,900	18,170	10.08
96	.82	142	160	168	157	15,700	19,146	10.62

* radioactivity in excess of background

VII EFFECT OF THE CONCENTRATION OF P^{32} IN THE GROWTH MEDIUM ON THE P^{32} RETENTION AND THE VIABLE CELL COUNT OF <u>SERRATIA MARCESCENS</u> DURING SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

TABLE VII a

MEASUREMENT OF RADIOACTIVITY IN THE GROWTH MEDIA

Flask number	μc P^{32}/75 ml medium	c/m* ml medium			Average c/m* ml medium	c/m* 75 ml medium
		1	2	3		
1	0	0	0	0	0	0
2	2.5	2,264	2,212	2,258	2,245	168,375
3	3.75	3,260	3,286	3,267	3,271	245,325
4	5.0	4,259	4,325	4,300	4,295	322,125
5	6.25	5,555	5,360	5,392	5,436	407,700
6	7.5	6,655	6,540	6,531	6,575	493,125
7	10.0	8,348	8,512	8,407	8,422	631,650
8	15.0	12,463	12,447	12,523	12,478	935,850
9	20.0	16,504	16,584	16,473	16,520	1,239,000

* radioactivity in excess of background.

TABLE VII b

MEASUREMENT OF THE AMOUNT OF P^{32} ASSIMILATED AND THE VIABLE CELL COUNT OF *SERRATIA MARCESCENS* AFTER 8 HOURS INCUBATION IN FLASKS IN A CONTROLLED TEMPERATURE WATER BATH SHAKER AT 30°C.

Flask number	Time factor	c/m* ml suspension			Average c/m* ml suspension	c/m* total suspension	Time corrected c/m* total suspension	Per cent P^{32} assimilation	Viable cell count/ml X 10^{-9}
		1	2	3					
1	.95	0	0	0	0	0	0	0	6.03
2	.95	784	754	910	816	61,200	64,421	38.36	6.12
3	.95	1265	1038	1429	1244	93,300	98,211	40.03	6.21
4	.95	1624	1463	1551	1546	115,950	122,052	37.89	6.17
5	.95	1948	1934	1986	1956	146,700	154,421	37.88	6.15
6	.95	2432	2264	2405	2367	177,525	186,868	37.89	6.09
7	.95	3087	3094	2852	3011	225,825	237,710	37.63	6.03
8	.95	4287	4488	4692	4489	336,675	354,395	37.87	5.91
9	.95	5902	5978	5946	5942	445,650	469,105	37.86	5.87

* radioactivity in excess of background.

TABLE VII c

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 12 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant			Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml X 10^{-9}
		1	2	3					
1	.90	0	0	0	0	0	0	0	4.75
2	.90	23	30	30	28	2,100	2,333	3.62	4.71
3	.90	37	33	48	39	2,925	3,250	3.31	4.51
4	.90	43	49	43	45	3,375	3,750	3.07	4.32
5	.90	71	62	61	65	4,875	5,417	3.51	4.21
6	.90	73	87	84	81	6,075	6,750	3.61	3.71
7	.90	117	121	97	112	8,400	9,333	3.93	3.64
8	.90	152	151	161	155	11,625	12,917	3.64	3.32
9	.90	230	213	238	227	17,025	18,917	4.03	3.51

* radioactivity in excess of background.

TABLE VII d

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 24 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant 1	2	3	Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml X 10^{-9}
1	.90	0	0	0	0	0	0	0	3.05
2	.90	31	37	31	33	2,475	2,750	4.27	3.12
3	.90	51	47	64	54	4,050	4,722	4.81	3.09
4	.90	67	69	58	65	4,875	5,417	4.44	2.91
5	.90	78	82	67	76	5,700	6,333	4.10	3.04
6	.90	101	91	125	106	7,950	8,833	4.73	2.58
7	.90	119	137	148	135	10,125	11,250	4.73	2.12
8	.90	161	153	171	162	12,150	13,444	3.79	2.03
9	.90	297	271	283	284	21,300	23,667	5.05	1.92

* radioactivity in excess of background.

TABLE VII e

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 36 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant			Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml X 10^{-9}
		1	2	3					
1	.86	0	0	0	0	0	0	0	2.43
2	.86	37	51	26	38	2,850	3,314	5.14	2.24
3	.86	51	67	51	56	4,200	4,884	4.97	2.35
4	.86	77	63	75	72	5,400	6,279	5.15	2.06
5	.86	83	91	94	89	6,675	7,762	5.03	2.03
6	.86	113	131	129	124	9,300	10,814	5.79	1.67
7	.86	147	157	145	150	11,250	13,081	5.50	1.24
8	.86	213	247	248	236	17,700	20,581	5.81	1.08
9	.86	319	346	361	342	25,650	29,826	6.36	1.14

* radioactivity in excess of background.

TABLE VII f

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 48 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant			Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml
		1	2	3					
1	.86	0	0	0	0	0	0	0	1.68×10^9
2	.86	46	38	35	40	3,000	3,488	5.42	1.63×10^9
3	.86	53	61	62	59	4,425	5,145	5.24	1.65×10^9
4	.86	71	73	80	75	5,625	6,541	5.36	1.29×10^9
5	.86	89	87	105	94	7,050	8,198	5.31	1.33×10^9
6	.86	133	137	115	128	9,600	11,163	5.97	9.6×10^8
7	.86	139	162	172	158	11,850	13,779	5.79	7.3×10^8
8	.86	233	267	243	248	18,600	21,628	6.10	6.8×10^8
9	.86	339	353	365	352	26,400	30,698	6.54	7.1×10^8

* radioactivity in excess of background.

TABLE VII g

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF *SERRATIA MARCESCENS* AFTER 60 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant			Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml
		1	2	3					
1	.82	0	0	0	0	0	0	0	1.35×10^9
2	.82	46	34	46	42	3,150	3,841	5.96	1.31×10^9
3	.82	49	79	59	62	4,650	5,671	5.77	1.10×10^9
4	.82	87	61	85	78	5,850	7,134	5.85	1.05×10^9
5	.82	93	102	100	98	7,350	8,963	5.80	1.04×10^9
6	.82	133	121	138	131	9,825	11,982	6.41	6.6×10^8
7	.82	153	167	171	164	12,300	15,000	6.31	4.7×10^8
8	.82	263	271	234	256	19,200	23,414	6.61	3.8×10^8
9	.82	346	386	352	361	27,075	33,018	7.04	4.1×10^8

* radioactivity in excess of background.

TABLE VII h

MEASUREMENT OF P^{32} LEACHED AND VIABLE CELL COUNT OF SERRATIA MARCESCENS AFTER 72 HOURS SUSPENSION IN DEMINERALIZED DISTILLED WATER AT 4°C.

Flask number	Time factor	c/m* ml suspension supernatant			Average c/m* ml suspension supernatant	c/m* total suspension supernatant	Time corrected c/m* total suspension supernatant	Per cent radio-activity leached	Viable cell count/ml X 10^{-8}
		1	2	3					
1	.82	0	0	0	0	0	0	0	9.2
2	.82	45	41	45	44	3,300	4,024	6.25	8.7
3	.82	65	69	63	66	4,950	6,037	6.15	8.4
4	.82	86	63	93	81	6,075	7,409	6.07	6.3
5	.82	99	109	97	102	7,650	9,329	6.04	6.5
6	.82	131	133	145	136	10,200	12,439	6.66	4.4
7	.82	171	167	178	172	12,900	15,732	6.62	2.71
8	.82	261	287	255	268	20,100	24,512	6.92	2.04
9	.82	389	386	352	376	28,200	34,390	7.33	2.15

* radioactivity in excess of background.

A P P E N D I X C

OPTICAL DENSITY READINGS OF THE 24 HOUR EXTRACTION SAMPLES TO DETERMINE COMPLETION OF EXTRACTION OF OIL FROM CORES FROM THE VARIOUS PETROLIFEROUS FORMATIONS.

CORES

Hours of Extraction	Pekisko 1	Pekisko 3	Pekisko 5	Pekisko 7	Pekisko 11	Pekisko 13	Cardium 1	Cardium 4	Beaverhill Lake 2	Beaverhill Lake 4	Belly River 5	Viking 2
0- 24	.647	1.08	.736	.936	1.012	.750	2.0	.397	.310	.430	.470	.310
24- 48	.536	.910	.430	.874	.598	.440	1.85	.260	.335	.080	.885	.337
48- 72	.217	.397	.360	1.014	.252	.325	1.08	.267	.210	.197	.593	.210
72- 96	.436	.267	.226	.335	.336	.252	.910	.250	.312	.200	.470	.312
96-120	.335	.273	.210	.436	.366	.192	.245	.273	.229	.262	.075	.229
120-144	.263	.245	.229	.391	.325	.165	.020	.272	.040	.175	.010	.040
144-168	.292	.312	.040	.420	.245	.138	-	.088	.090	.090	-	-
168-192	.217	.229	.197	.210	.222	.163		-	-	.030		
192-216	.163	.167	.090	.322	.325	.226				-		
216-240	.284	.070	.031	.243	.263	.173						
240-264	.216	.163	.047	.229	.190	.140						
264-288	.092	.060	-	.136	.183	.169						
288-312	.117	.015		.065	.136	.132						
312-336	.132	-		.040	.132	.138						
336-360	.054			-	.063	.072						
360-384	-				.030	-						
384-408					-							

APPENDIX D

Example calculation of the amount of leached P^{32} which could be present in a core volume having 10% effective porosity over a 48 hour period if the labelled cells had originally assimilated 40% of the available P^{32} and leached 8% of the label during the 48 hour period.

Initial P^{32} concentration in medium = 7.5 μc P^{32}/150 ml

If 40% available P^{32} assimilated by cells, then there is 3.0 μc P^{32}/cells.

The labelled cells are then suspended in 130 ml water.

If 8% of the assimilated P^{32} is leached, (.08 X 3.0 = 0.24 μc P^{32}), into the supernatant, there are 0.24 μc P^{32}/130 ml slurry or 0.24/130 = 0.0018 μc P^{32}/ml supernatant.

From the extrapolation in Figure 7, 0.48 cm is the maximal thickness of core material which allows the passage of Beta particles.

Volume of a cube of core having dimensions (1 cm X 1 cm X 0.48 cm) = 0.48 cc.

Effective pore space of above cube of core, if effective porosity is 10% = 0.1 X 0.48 = 0.048 cc.

If 8% of assimilated P^{32} is leached, then the amount of leached P^{32} which could be present in the pore space of above cube of core

= 0.048 cc X 0.0018 μc P^{32}

= 0.0000864 μc P^{32}.

APPENDIX E

Detailed study of the migration of labelled Serratia marcescens through oil extracted and non-extracted cores from the different petroliferous formations.

I DETAILS OF MIGRATION STUDIES WITH CORES FROM TWINNING FIELD, PEKISKO FORMATION.

TABLE I a

MEASUREMENT OF P^{32} AVAILABLE IN MEDIA

Slurry for Core No.	c/m* ml media 1	2	3	Average c/m* ml	c/m* 150 ml
1	4633	4618	4785	4697	701,850
2	4433	4452	4605	4497	674,550
3	4440	4509	4673	4541	681,150
4	4484	4763	4562	4603	690,450
5	4669	4704	4653	4675	701,250
6	4044	4138	4158	4113	616,950
7	4484	4503	4405	4464	669,600
8	4052	3998	3970	4007	601,050
9	4200	4267	4292	4253	637,950
10	4872	4823	4712	4802	720,300
11	4636	4354	4641	4544	681,600
12	4822	4878	4665	4788	718,200
13	4539	4684	4533	4585	687,750
14	4456	4448	4401	4435	665,250
15	4567	4522	4450	4513	676,950

* radioactivity in excess of background

TABLE I b

MEASUREMENT OF P^{32} ASSIMILATED BY CELLS

Core No.	Decay Factor	c/m* ml slurry			Average c/m* ml	c/m* 130 ml	c/m* 130 ml time corrected	Assimi-lation of P^{32}	Viable cell count/ml (X 10^{-9})	Viable count of total volume of slurry (X 10^{-9})	c/m* 10^9 cells
		1	2	3							
1	1.0	2438	2482	2502	2474	321,620		45.82	8.8	1144	281.14
2	.94	2380	2256	2269	2302	299,260	318,362	47.19	9.2	1196	266.19
3	1.0	2675	2579	2509	2588	336,440		49.39	9.4	1222	275.32
4	.54	1195	1256	1247	1233	160,290	296,833	42.99	8.3	1079	275.10
5	1.0	2605	2716	2641	2654	345,020		49.20	9.3	1209	285.38
6	.865	1833	1835	1824	1831	238,030	275,179	44.60	8.2	1066	258.14
7	1.0	2762	2388	2381	2510	326,300		48.72	9.1	1183	275.83
8	.865	1563	1696	1674	1644	213,720	247,075	41.11	7.6	988	250.08
9	.91	1888	1792	1857	1846	239,980	263,714	41.34	7.3	949	277.89
10	.875	1784	1789	1775	1783	231,790	264,903	36.77	7.6	988	268.12
11	1.0	2097	2099	2139	2112	274,560		40.28	7.5	975	281.60
12	.875	1824	1803	1763	1797	233,610	266,983	37.17	7.4	962	277.53
13	1.0	1849	2016	2163	2009	261,170		37.97	7.6	988	264.34
14	1.0	2288	2269	2287	2281	296,530		44.57	7.9	1027	288.73
15	1.0	2207	2307	2318	2277	296,010		43.73	8.1	1053	281.11

* radioactivity in excess of background.

TABLE I c

MEASUREMENT OF P^{32} REMAINING IN THE SLURRY AFTER THE MIGRATION HAD PROCEEDED 48 HOURS

Core No.	Decay Factor	mls slurry 0 hours	mls slurry 48 hours	c/m* ml slurry 1	2	3	Average c/m* ml	c/m* slurry 48 hours	c/m* slurry 48 hours time corrected
1	.9	75	74	2216	2173	2219	2203	163,022	181,135
2	.9	125	124	2269	2113	2236	2206	273,544	303,938
3	.9	75	74	2202	2408	2376	2329	172,346	191,495
4	.54	125	124	1218	1211	1263	1231	152,644	282,674
5	.87	70	67	2168	2406	2283	2286	153,162	176,048
6	.865	125	124	1788	1847	1863	1833	227,292	262,765
7	.87	70	68	2105	2207	2236	2183	148,444	170,625
8	.865	125	124	1677	1629	1631	1646	204,104	235,958
9	.91	65	63	1864	1849	1963	1892	119,196	130,984
10	.82	125	124	1735	1649	1643	1676	207,824	253,444
11	.834	70	70	1866	1613	1783	1754	122,780	147,218
12	.82	125	124	1635	1706	1733	1691	209,684	255,712
13	.834	70	70	1603	1693	1713	1670	116,900	140,168
14	.9	70	70	2227	1891	1976	2031	142,170	157,967
15	.9	70	70	1937	2217	1944	2033	142,310	158,122

* radioactivity in excess of background.

TABLE I d

MEASUREMENT OF P^{32} LEACHED FROM THE CELLS IN THE SLURRY DURING PERIOD OF MIGRATION

(48 HOURS DURATION)

Core No.	Decay Factor	c/m* ml supernatant			Average c/m* ml supernatant	c/m* total supernatant	c/m* total supernatant time corrected	Per cent P^{32} leached
		1	2	3				
1	.9	177	166	166	170	12,750	14,167	7.63
2	.9	148	164	163	158	19,750	21,944	7.18
3	.9	193	185	189	189	14,175	15,750	8.11
4	.54	95	103	106	101	12,624	23,379	8.20
5	.87	159	181	193	178	12,460	14,322	7.71
6	.865	132	151	156	146	18,250	21,098	7.99
7	.87	191	161	151	168	11,760	13,517	7.69
8	.865	136	137	139	137	17,125	19,798	8.35
9	.91	146	129	126	134	8,710	9,571	7.26
10	.82	123	106	133	121	15,125	18,445	7.25
11	.834	112	135	136	128	8,960	10,743	7.27
12	.82	141	106	133	127	15,875	19,360	7.55
13	.834	159	139	103	134	9,380	11,247	8.00
14	.9	162	152	191	168	11,760	13,066	8.18
15	.9	153	162	155	157	10,990	12,211	7.66

* radioactivity in excess of background.

TABLE I e

SUMMARY OF MIGRATION STUDIES THROUGH TWINNING FIELD, PEKISKO FORMATION CORES.

Core No.	mls of slurry migrated	c/m* slurry 0 hours	c/m* slurry 48 hours	c/m* migrated	Total possibly migrated as leach c/m*	Total possibly migrated as leach time corrected c/m*	Total migrated as cells c/m*	Equivalent number of cells (X 10-9)
1	1	185,550	181,135	4415	170	189	4226	15.03
2	1	305,627	303,938	1689	158	175	1514	5.69
3	1	194,100	191,495	2605	189	210	2395	8.70
4	1	284,960	282,674	2286	101	187	2099	7.63
5	3	185,780	176,048	9732	534	614	9118	31.95
6	1	264,172	262,765	1407	146	169	1238	4.80
7	2	175,700	170,625	5075	336	386	4689	17.00
8	1	237,192	235,958	1234	137	158	1076	4.30
9	2	131,857	130,984	873	268	295	578	2.08
10	1	254,307	253,444	863	121	148	715	2.67
11	0	147,840	147,218	622	0	0	622	2.21
12	1	256,304	255,712	592	127	155	437	1.57
13	0	140,630	140,168	462	0	0	462	1.75
14	0	159,670	157,967	1703	0	0	1703	5.89
15	0	159,390	158,122	1268	0	0	1268	4.51

* radioactivity in excess of background.

TABLE I f

RADIOACTIVITY AT THE SPLIT SURFACE OF TWINNING FIELD, PEKISKO FORMATION CORES.

Inches from slurry end	Core Number 1 c/m*	3 c/m*	5 c/m*	7 c/m*	11 c/m*	13 c/m*
0.5	87	20	14	0	23	31
1.25	15	7	0	0	0	0
2.0	7	0	0	0	0	0
2.75	10	0	0	0	0	0

* radioactivity in excess of background.

II DETAILS OF MIGRATION STUDIES WITH CORES FROM PEMBINA FIELD, CARDIUM FORMATION.

TABLE II a

MEASUREMENT OF P^{32} AVAILABLE IN MEDIA

Slurry for Core No.	c/m* ml media 1	2	3	Average c/m* ml	c/m* 150 ml
1	4552	4513	4497	4521	678,150
2	4574	4424	4620	4539	680,850
3	3901	4195	4050	4049	607,350
4	3789	3838	3799	3809	571,350

* radioactivity in excess of background.

TABLE II b

MEASUREMENT OF P^{32} ASSIMILATED BY CELLS

Core No.	Decay Factor	c/m* ml slurry 1	2	3	Average c/m* ml	c/m* 130 ml	c/m* 130 ml time corrected	Assimi-lation of P^{32}	Viable cell count/ml (X 10^{-9})	Viable count of total volume of slurry	c/m* 10^9 cells
1	.96	2488	2427	2484	2466	320,580	333,938	49.16	8.0	1040	321.09
2	.96	2389	2512	2385	2429	315,770	328,927	48.31	8.6	1118	294.21
3	1.0	1814	1752	1789	1785	232,050		38.21	6.9	897	258.59
4	1.0	1620	1635	1611	1622	210,860		36.91	6.8	884	238.53

* radioactivity in excess of background.

TABLE II c

MEASUREMENT OF P^{32} REMAINING IN THE SLURRY AFTER THE MIGRATION HAD PROCEEDED 48 HOURS

Core No.	Decay Factor	mls slurry 0 hours	mls slurry 48 hours	c/m* ml slurry 1	2	3	Average c/m* ml	c/m* slurry 48 hours	c/m* slurry 48 hours time corrected
1	.865	75	74	2247	2281	2210	2246	166,204	192,143
2	.865	75	74	2245	2186	2093	2175	163,125	188,584
3	.905	75	74	1688	1673	1533	1631	120,694	133,363
4	.905	75	74	1569	1463	1427	1486	109,964	121,507

* radioactivity in excess of background.

TABLE II d

MEASUREMENT OF P^{32} LEACHED FROM THE CELLS IN THE SLURRY DURING PERIOD OF MIGRATION (48 HOURS DURATION).

Core No.	Time Factor	c/m* ml supernatant 1	2	3	Average c/m* ml supernatant	c/m* total supernatant	c/m* total supernatant time corrected	Per cent P^{32} leached
1	.865	158	179	156	164	12,300	14,219	7.38
2	.865	168	162	150	160	12,000	13,873	7.31
3	.905	95	103	94	97	7,275	8,039	6.00
4	.905	97	80	89	89	6,675	7,376	6.06

* radioactivity in excess of background.

TABLE II e

SUMMARY OF MIGRATION STUDIES THROUGH PEMBINA FIELD, CARDIUM FORMATION CORES.

Core No.	mls of slurry migrated	c/m* slurry 0 hours	c/m* slurry 48 hours	c/m* migrated	Total possibly migrated as leach c/m*	Total possibly migrated as leach time corrected c/m*	Total migrated as cells c/m*	Equivalent number of cells (X 10^{-9})
1	1	192,682	192,143	539	164	190	349	1.09
2	1	189,791	188,584	1022	160	185	837	2.84
3	1	133,875	133,363	512	97	107	405	1.57
4	1	121,650	121,507	143	89	98	45	.19

* radioactivity in excess of background.

TABLE II f

RADIOACTIVITY AT THE SPLIT SURFACE OF PEMBINA FIELD, CARDIUM FORMATION CORES

Inches from slurry end	Core Number 1 c/m*	2 c/m*	3 c/m*	4 c/m*
0.5	22	0	0	0
1.25	0	0	0	0
2.0	0	0	0	0
2.75	0	0	0	0

* radioactivity in excess of background.

III DETAILS OF MIGRATION STUDIES WITH CORES FROM SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION.

TABLE III a

MEASUREMENT OF P^{32} AVAILABLE IN MEDIA

Slurry for core No.	c/m* ml media 1	2	3	Average c/m* ml	c/m* 150 ml media
1	5261	5253	5132	5215	782,250
2	5086	5093	4905	5028	754,200
3	3842	4144	4037	4008	601,200
4	3971	4106	4143	4073	610,950
5	5269	5319	5341	5310	796,500
6	4967	5077	4901	4982	747,300

* radioactivity in excess of background.

TABLE III b

MEASUREMENT OF P^{32} ASSIMILATED BY CELLS

Core No.	Decay Factor	c/m* ml slurry 1	2	3	Average c/m* ml slurry	c/m* 130 ml slurry	Per cent P^{32} assimi-lation	Viable cell count/ml ($x 10^{-9}$)	Viable count of total volume of slurry ($x 10^{-9}$)	c/m* 10^9 cells
1	1.0	2463	2520	2439	2474	321,620	41.12	8.6	1118	287.67
2	1.0	2631	2512	2601	2581	335,530	44.49	8.8	1144	293.29
3	1.0	1562	1552	1536	1550	201,500	33.52	6.11	794	253.78
4	1.0	1723	1888	1797	1803	234,390	38.36	6.3	819	286.19
5	1.0	2445	2456	2421	2441	317,330	39.84	7.75	1007	315.12
6	1.0	2151	2134	2198	2161	280,930	37.59	7.6	988	284.34

* radioactivity in excess of background.

TABLE III c

MEASUREMENT OF P^{32} REMAINING IN THE SLURRY AFTER THE MIGRATION HAD PROCEEDED 48 HOURS

Core No.	Decay Factor	mls slurry 0 hours	mls slurry 48 hours	c/m* ml slurry 48 hours 1	2	3	Average c/m* ml slurry	c/m* slurry 48 hours	c/m* slurry 48 hours time corrected
1	.91	75	71	2315	2196	2191	2234	158,614	174,301
2	.91	75	72	2436	2295	2447	2393	172,296	189,336
3	.87	75	74	1307	1373	1383	1354	100,196	115,168
4	.87	75	71	1564	1578	1612	1585	112,535	129,351
5	.90	75	74	2235	2221	2212	2223	164,502	182,780
6	.90	75	71	2021	1987	1998	2002	142,142	157,935

* radioactivity in excess of background.

TABLE III d

MEASUREMENT OF P^{32} LEACHED FROM THE CELLS IN THE SLURRY DURING PERIOD OF MIGRATION

(48 HOURS DURATION)

Core No.	Decay Factor	c/m* ml supernatant 1	2	3	Average c/m* ml supernatant	c/m* total supernatant	c/m* total supernatant time corrected	Per cent P^{32} leached
1	.91	174	163	181	173	12,975	14,258	7.68
2	.91	173	190	204	189	14,175	15,577	8.05
3	.87	112	105	136	118	8,850	10,172	8.75
4	.87	140	98	139	126	9,450	10,862	8.03
5	.90	171	153	198	174	13,050	14,500	7.92
6	.90	194	195	193	194	14,550	16,166	9.97

* radioactivity in excess of background.

TABLE III e

SUMMARY OF MIGRATION STUDIES THROUGH SWAN HILLS FIELD, BEAVERHILL LAKE FORMATION CORES.

Core No.	mls of slurry migrated	c/m* slurry 0 hours	c/m* slurry 48 hours	c/m* migrated	Total possibly migrated as leach c/m*	Total possibly migrated as leach time corrected c/m*	Total migrated as cells c/m*	Equivalent number of cells (X 10^{-9})
1	4	185,550	174,301	11,249	692	760	10,489	36.46
2	3	193,575	189,336	4,239	567	623	3,616	12.33
3	1	116,250	115,168	1,082	118	136	946	3.73
4	4	135,225	129,351	5,874	504	579	5,295	18.50
5	1	183,075	182,780	295	174	193	102	.32
6	4	162,075	157,935	4,140	776	862	3,278	11.53

* radioactivity in excess of background.

TABLE III f

RADIOACTIVITY AT THE SPLIT SURFACE OF CORES, (SWAN HILLS FIELD BEAVERHILL LAKE FORMATION)

Inches from slurry end	Core Number 1 c/m*	2 c/m*	3 c/m*	4 c/m*	5 c/m*	6 c/m*
0.5	54	55	15	26	4	81
1.25	0	9	11	5	12	7
2.0	0	0	0	4	0	10
2.75	0	0	0	0	0	0

* radioactivity in excess of background.

IV DETAILS OF MIGRATION STUDIES WITH CORES FROM WILLESDEN GREEN FIELD, BELLY RIVER FORMATION.

TABLE IV a

MEASUREMENT OF P^{32} AVAILABLE IN MEDIUM

Slurry for Core No.	c/m* ml medium 1	2	3	Average c/m* ml	c/m* 150 ml medium
1	4355	4423	4406	4395	659,250
2	4357	4343	4301	4334	650,100
3	5053	5093	4913	5020	753,000
4	5131	5081	5154	5122	768,300
5	4350	4346	4228	4308	646,200

* radioactivity in excess of background.

TABLE IV b

MEASUREMENT OF P^{32} ASSIMILATED BY CELLS

Core No.	Decay Factor	c/m* ml slurry 1	2	3	Average c/m* ml slurry	c/m* 130 ml slurry	c/m* 130 ml time corrected	Per cent P^{32} assimi-lation	Viable cell count/ml ($X 10^{-9}$)	Viable count of total volume of slurry ($X 10^{-9}$)	c/m* 10^9 cells
1	.95	1876	1915	1896	1896	246,480	259,453	39.36	7.3	949	273.40
2	.95	1951	1882	1863	1899	246,870	259,863	39.97	7.5	975	266.53
3	1.0	2563	2532	2586	2560	332,800		44.19	8.6	1118	297.67
4	1.0	2838	2677	2774	2763	359,190		46.75	8.9	1157	310.45
5	1.0	1928	1913	1838	1893	246,090		38.08	7.1	923	266.62

* radioactivity in excess of background.

TABLE IV c

MEASUREMENT OF P^{32} REMAINING IN THE SLURRY AFTER THE MIGRATION HAD PROCEEDED 48 HOURS.

Core No.	Decay Factor	mls slurry 0 hours	mls slurry 48 hours	c/m* ml slurry 48 hours 1	2	3	Average c/m* ml slurry	c/m* slurry 48 hours	c/m* slurry 48 hours time corrected
1	.824	120	116	1714	1593	1678	1662	192,792	233,971
2	.824	120	119	1622	1566	1755	1648	196,112	238,000
3	.9	75	71	2276	2291	2316	2294	162,874	180,971
4	.9	75	69	2497	2464	2436	2466	170,154	189,060
5	.9	75	74	1571	1793	1746	1703	126,022	140,024

* radioactivity in excess of background.

TABLE IV d

MEASUREMENT OF P^{32} LEACHED FROM THE CELLS IN THE SLURRY DURING PERIOD OF MIGRATION.

(48 HOURS DURATION)

Core No.	Decay Factor	c/m* ml supernatant 1	2	3	Average c/m* ml supernatant	c/m* total supernatant	c/m* total supernatant time corrected	Per cent P^{32} leached
1	.824	117	129	131	126	15,120	18,349	7.69
2	.824	122	126	137	128	15,360	18,641	7.80
3	.9	171	163	191	175	13,125	14,583	7.60
4	.9	177	187	193	186	13,950	15,500	7.48
5	.9	124	130	131	128	9,600	10,667	7.51

* radioactivity in excess of background.

TABLE IV e

SUMMARY OF MIGRATION STUDIES THROUGH WILLESDEN GREEN FIELD, BELLY RIVER FORMATION CORES.

Core No.	mls of slurry migrated	c/m* slurry 0 hours	c/m* slurry 48 hours	c/m* migrated	Total possibly migrated as leach c/m*	Total possibly migrated as leach time corrected c/m*	Total migrated as cells c/m*	Equivalent number of cells (x 10^{-9})
1	4	238,697	233,971	4,726	504	612	4,114	15.05
2	1	239,074	238,000	1,074	128	155	919	3.45
3	4	192,000	180,971	11,029	700	778	10,251	34.44
4	6	207,225	189,060	18,165	1,116	1,240	16,925	54.52
5	1	141,975	140,024	1,951	128	142	1,809	6.78

* radioactivity in excess of background.

TABLE IV f

RADIOACTIVITY AT THE SPLIT SURFACE OF WILLESDEN GREEN FIELD, BELLY RIVER FORMATION CORES.

Inches from slurry end	Core number 3 c/m*	Core number 4 c/m*	Core number 5 c/m*
0.5	266	344	29
1.25	42	54	0
2.0	31	8	0
2.75	11	6	0

* radioactivity in excess of background.

V DETAILS OF MIGRATION STUDIES WITH CORES FROM BEAVERHILL LAKE FIELD, VIKING FORMATION.

TABLE V a

MEASUREMENT OF P^{32} AVAILABLE IN THE MEDIUM

Slurry for Core No.	c/m* ml medium 1	2	3	Average c/m* ml	c/m* 150 ml medium
1	5065	5076	5172	5104	765,600
2	4227	4146	4236	4203	630,450
3	5216	5151	5076	5148	772,200
4	4071	4138	4141	4117	617,550
5	4437	4466	4454	4452	667,800

* radioactivity in excess of background.

TABLE V b

MEASUREMENT OF P^{32} ASSIMILATED BY CELLS.

Core No.	Decay Factor	c/m* ml slurry 1	2	3	Average c/m* ml slurry	c/m* 130 ml slurry	c/m* 130 ml slurry time corrected	Per cent P^{32} assimilation	Viable cell count/ml slurry (X 10^{-9})	Viable count of total volume of slurry (X 10^{-9})	c/m* 10^9 cells
1	.95	2579	2703	2661	2648	344,240	362,358	47.33	9.8	1274	284.43
2	1.0	2016	2120	2038	2058	267,540		42.44	7.1	923	289.86
3	.95	2339	2769	2554	2554	332,020	349,495	45.26	9.25	1203	290.64
4	1.0	2061	1981	2028	2023	262,990		42.58	7.3	949	277.12
5	1.0	1973	2042	2008	2008	261.040		39.09	7.8	1014	257.44

* radioactivity in excess of background.

TABLE V c

MEASUREMENT OF P^{32} REMAINING IN THE SLURRY AFTER THE MIGRATION HAD PROCEEDED 48 HOURS

Core No.	Decay Factor	mls slurry 0 hours	mls slurry 48 hours	c/m* ml slurry 48 hours 1	2	3	Average c/m* ml slurry	c/m* slurry 48 hours	c/m* slurry 48 hours time corrected
1	.9	125	122	2516	2491	2546	2518	307,196	341,329
2	.9	75	70	1811	1926	1893	1877	131,390	145,990
3	.9	125	120.5	2274	2512	2494	2427	292,453	324,948
4	.9	75	70.5	1782	1978	1833	1864	131,412	146,013
5	.9	75	72	1754	1838	1845	1812	130,464	144,960

* radioactivity in excess of background.

TABLE V d

MEASUREMENT OF P^{32} LEACHED FROM THE CELLS IN THE SLURRY DURING PERIOD OF MIGRATION (48 HOURS DURATION).

Core No.	Decay Factor	c/m* ml supernatant 1	2	3	Average c/m* ml supernatant	c/m* total supernatant	c/m* total supernatant time corrected	Per cent P^{32} leached
1	.9	206	222	216	215	26,875	29,861	8.58
2	.9	123	151	163	146	10,950	12,167	7.88
3	.9	156	201	245	201	25,125	27,917	8.32
4	.9	158	113	127	133	9,975	11,083	7.30
5	.9	123	167	132	141	10,575	11,750	7.80

* radioactivity in excess of background.

TABLE V e

SUMMARY OF MIGRATION STUDIES THROUGH BEAVERHILL LAKE FIELD, VIKING FORMATION CORES.

Core No.	mls of slurry migrated	c/m* slurry 0 hours	c/m* slurry 48 hours	c/m* migrated	Total possibly migrated as leach c/m*	Total possibly migrated as leach time corrected c/m*	Total migrated as cells c/m*	Equivalent number of cells (X 10^{-10})
1	3	347,864	341,329	6,535	645	717	5,818	2.05
2	5	154,350	145,990	8,360	730	811	7,549	2.60
3	4.5	335,515	324,948	10,567	904	1,004	9,563	3.29
4	4.5	151,725	146,013	5,712	598	664	5,048	1.82
5	3.0	150,600	144,960	5,640	423	470	5,170	2.01

* radioactivity in excess of background.

TABLE V f

RADIOACTIVITY AT THE SPLIT SURFACE OF BEAVERHILL LAKE FIELD, VIKING FORMATION CORES.

Inches from slurry end	2 c/m*	4 c/m*	5 c/m*
0.5	63	178	66
1.25	10	37	0
2.0	5	5	0
2.75	0	0	0

* radioactivity in excess of background.

APPENDIX F

ANALYSES FOR EXPANDABLE CLAY CONTENT IN THE DIFFERENT FORMATIONS

Packed Slide-Gross Sample	Oriented Slide, - 10 Micron
Cardium Formation, Core #2	
85-90% Quartz	35-40% Quartz
5% Feldspar, near orthoclase	35-40% Kaolin
less than 5% Kaolin	5% Illite
1- 2% Anhydrite	5-10% Material X (see below)
less than 5% Illite-montmorillonite mixed layer material	less than 5% Montmorillonite

Material X may be either a beta-alumina, or a regular clay mineral of alternate illite and chlorite layers. It is completely unaffected by glycol treatment. The montmorillonite shows slight expansion with glycol, but less than normal for a swelling montmorillonite.

Beaverhill Lake, Core #2	
60-70% Calcite	60-70% Calcite
30-40% Dolomite	30-40% Dolomite
approx. 3% Quartz	trace Illite, not expandable
No evidence of clay minerals	trace Montmorillonite, expandable
Pekisko Formation, Core #6	
Major Calcite	Very small amount in this size range
1- 2% Dolomite	
2- 3% Quartz	80% Calcite
No evidence of clay minerals	20% Kaolin
	No evidence of other clays

Belly River Formation, Core #4, -- Contains considerable organic matter.

55-60% Quartz
20-25% Unidentified material (see below)
10% Kaolin
10-15% Illite plus mixed layer

30-35% Quartz
20-25% Kaolin
20-30% Illite plus mixed layer (slightly expandable)
20-30% Montmorillonite - normal expansion

The unidentified material appears to be an iron silicate, possibly hydrated. It does not appear in the -10 micron fraction, and is probably not a clay mineral.

Viking Formation, Core #5

40-45% Quartz
20-25% Unidentified material (see below)
20-25% Illite-montmorillonite mixed layer
10% Illite
5% Kaolin
less than 5% Chlorite

20-25% Quartz
20-25% Kaolin
25-30% Illite
25-30% Mixed layer material
approx. 5% Chlorite

This sample contains the same unidentified material as the Belly River Formation, also the same type of organic matter. There is a broad band on the pattern of the gross sample, extending from illite to montmorillonite. There is a small sharp chlorite peak in the -10 micron fraction, which is not altered by glycol. The mixed layer material expands from near illite to montmorillonite and somewhat beyond.

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